

Gateways to the underworld: molecular phylogenetic analyses identify patterns of groundwater fauna and two new species of hypogean *Hyaella* Smith, 1874 (Amphipoda, Hyaellidae) from the northern Mojave Desert

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Abstract

In North America, the genus *Hyaella* (Crustacea: Amphipoda) is primarily epigean, with only two described species found in groundwater. In contrast, *Stygobromus* is an amphipod genus occupying subterranean habitats throughout most of the Nearctic. Using both molecular and morphological traits, we describe two new species of stygobitic amphipods, *Hyaella plutonia* **sp. nov.** and *Hyaella keepuikantun* **sp. nov.** from groundwater habitats in Nye County, Nevada, USA. These species form a monophyletic group that also contains the similar *H. cretae* from Ash Meadows National Wildlife Refuge. Members of this “*H. cretae*” complex are similar in terms of overall morphology but can be distinguished through examination of taxonomically relevant characters within the genus such as the third uropod, gnathopods, and pereopod 7. The description of these species highlights the unexpected diversity of stygobitic *Hyaella* within the northern Mojave Desert, where four such species comprise two independent lineages. *Stygobromus* is absent from these locations. This diversity of *Hyaella* is hypothesized to be due in part to the evolutionary history the genus and the ecological/climatic conditions of the region itself, which may exclude other more wide-ranging Nearctic stygobites like *Stygobromus*.

Keywords

Devils Hole, *Hyaella muerta*, *Hyaella cretae*, Nevada

Introduction

While environments such as ponds, lakes, and streams are most commonly envisioned when considering freshwater on Earth, these habitats make up less than one percent of the Earth's freshwater; nearly 70% is contained in glaciers, permafrost, and ice caps, and the remaining ~30% exists as groundwater (Gleick 1993). Similar trends follow the fauna associated with freshwaters: common surface inhabitants of familiar, accessible, systems come to mind (sportfish, insects and insect larvae such as the “EPT taxa”, etc.) when considering “freshwater fauna”, but a significant number of taxa occupy groundwater systems as well. Many of the latter—including vertebrates such as salamanders and fishes, and invertebrates including Mollusca, Arthropoda, Annelida, Platyhelminthes, and other phyla—have been recorded in hypogean environments; in fact, these taxa often display remarkably high species richness (Stoch 1995; Danielpol et al. 2000; Maurice and Bloomfield 2012). Such diversity within these environments is often promoted by their nature, as predicted by the “adaptive zone model” (Stoch 1995). This model highlights the high potential for colonization to occur in hypogean systems which can then lead to speciation and often radiation; this may, in part, explain the high species diversity observed among crustacean taxa in several groundwater systems (Stoch 1995; Danielpol et al. 2000).

In the Nearctic, many taxa have been recorded from hypogean systems, but perhaps some of the highest such biodiversity can be observed within the Crustacea Brünnich, 1772, especially the abundant and species-rich members of the peracarid order Amphipoda Latreille, 1816 (Stoch 1995). To date, over 200 species from 19 genera and 8 families of amphipods have been identified from groundwater habitats in the Nearctic (Horton et al. 2025). A majority of these species are contained within families/genera which could be considered groundwater-obligates, such as the genus *Stygobromus* Cope, 1872 and most genera of the family Hadziidae S. Karaman, 1943 which do not possess surface representatives (Holsinger 1986; Horton et al. 2025). These taxa typically display highly stygomorphic facies and often represent older, often relictual, lineages (Holsinger 1986). Conversely, a smaller subset of taxa possesses both epigean and hypogean representatives; such a distribution is likely due to more recent introductions to groundwater. Examples of such taxa are found in the genera *Gammarus* Fabricius, 1775 and *Hyaletella* Smith, 1874 (Holsinger 1986; Cannizzaro et al. 2023; Horton et al. 2025).

Hyaletella is a primarily epigean genus endemic to the New World, with over 100 species currently described (Horton et al. 2025). The center of diversity for the genus is in the Neotropical realm, where an overwhelming majority (~80%) of species occur (De Los Ríos et al. 2012; Horton et al. 2025). Here, species have been recorded from a wide range of epigean (springs, swamps, lakes, streams, etc.) and hypogean (caves, springs, hyporheic habitats) habitats. While a number of *Hyaletella* spp. have been recorded from a variety of groundwater habitats in the Neotropics, hypogean *Hyaletella* are only known from spring habitats in the Great Basin of California and Nevada within the Nearctic. Two species have recently been described: *Hyaletella muerta* Baldinger, Shepard & Therooff, 2000 from Inyo County, California and *Hyaletella cretae* Cannizzaro, Lange, & Berg, 2023 from Nye County, Nevada. The presence of *Hyaletella* in some Nearctic groundwa-

ters itself is curious, as these habitats are mostly occupied by members of the genus *Stygobromus* (Holsinger 1986). While *Stygobromus* spp. are present in the Great Basin, they are not sympatric with *Hyaella* spp. and are absent throughout the Mojave Basin and Range and most of the Sonoran Desert. Furthermore, molecular phylogenetic analyses of *H. muerta* and *H. cretae* suggest while similar morphologically and separated by an overland distance of ~45 km, the two species are not sister taxa and belong to different lineages within the genus, likely colonizing groundwater habitats independently (Cannizzaro et al. 2023). The presence of multiple *Hyaella* lineages in groundwater within the region and the absence of *Stygobromus* spp. suggest that *Hyaella* were able to exploit groundwater habitats here that are not occupied by other amphipod taxa (Cannizzaro et al. 2023). As a result, it is likely that within the Mojave Basin and Range *Hyaella* spp. would follow diversification trends similar to those of other groundwater taxa such as *Stygobromus*, where different localities would contain isolated, endemic species.

Collections from Nye County, Nevada, USA have revealed the presence of additional populations of stygobitic *Hyaella* (Fig. 1). Here we examine these populations and similar species using both morphological analyses and molecular genetic comparisons of three genes commonly assessed for amphipod molecular phylogenetics: nuclear 18S ribosomal DNA (Cannizzaro et al. 2023; Cannizzaro et al. 2025b), nuclear 28S ribosomal DNA (Witt et al. 2006; Hou et al. 2007; Flot et al. 2010; Cannizzaro et al. 2023; Cannizzaro et al. 2025a, 2025b), and mitochondrial 16S rDNA (Macdonald et

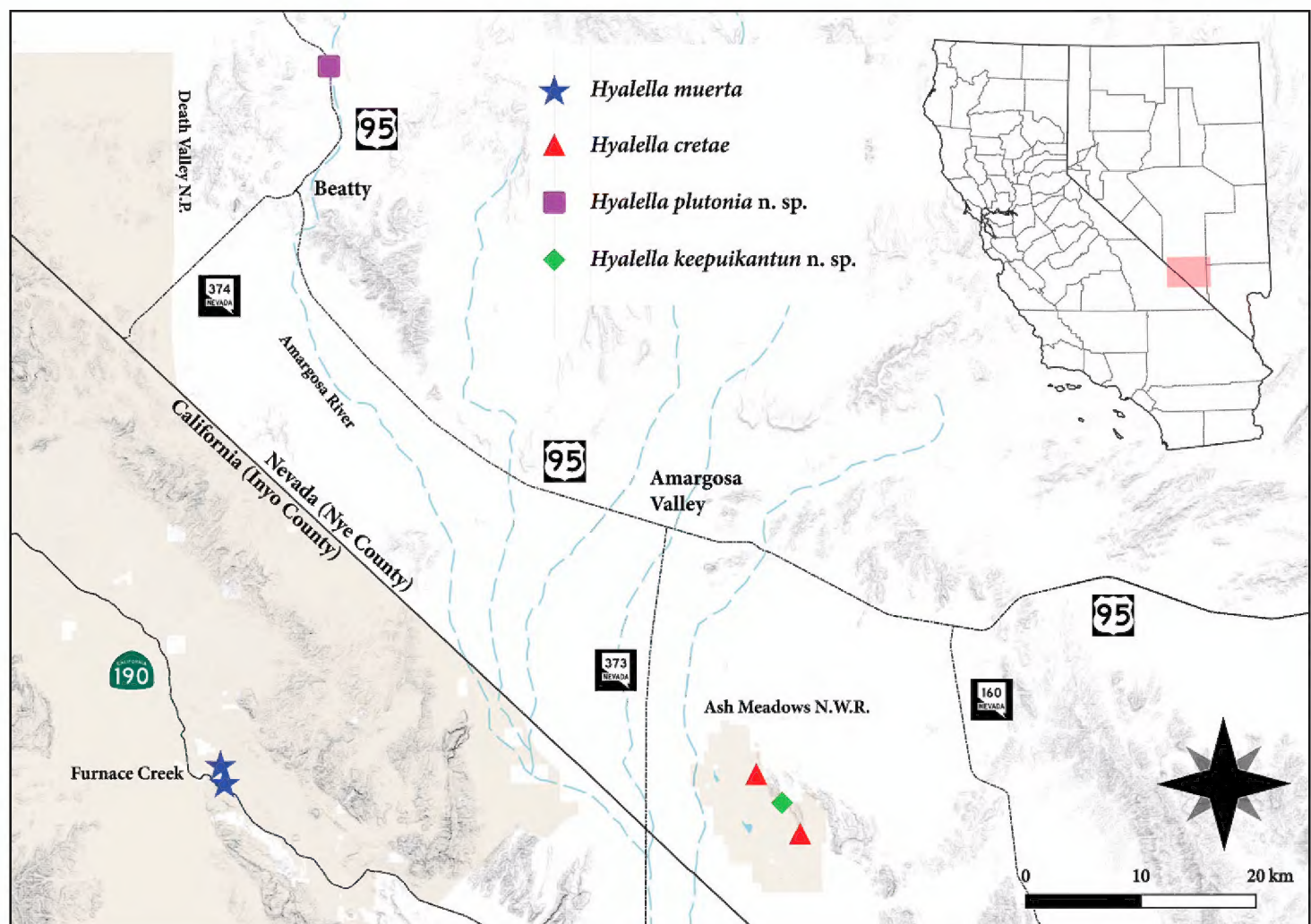


Figure 1. Updated distributions of hypogean *Hyaella* spp. in Nye County, Nevada and Inyo County, California, USA.

al. 2005; Hou et al. 2007; Cannizzaro et al. 2023; Cannizzaro et al. 2025a, 2025b). The mitochondrial cytochrome c oxidase subunit I (COI) gene which is also often assessed was excluded from these analyses due to issues with pseudogenes.

Materials and methods

Collection of specimens

Individuals were collected live, using hand nets or small strainers, from both an unnamed spring, ~0.7 km north of the Highway 95 and Oleo Rd intersection near Beaty, and Devils Hole, Nye County, Nevada (Fig. 1). Collected specimens were preserved in 95% ethanol and stored at -20 °C. Specimens examined here were deposited at the National Museum of Natural History, Smithsonian Institution (**USNM**).

Morphological analyses

To aid in morphological analysis, four specimens of each population were cleared and stained prior to dissection. These individuals were digested in a mixture of 400 µL of Molecular Grade Water, 360 µL of 2× Digestion Buffer (Zymo Research, Irvine, CA, USA), and 40 µL of Proteinase K; samples were incubated in this mixture at 37 °C for 2 hours and then at room temperature overnight. Once sufficiently digested, specimens were transferred to a solution of Lignin Pink for 2–3 hours; after specimens were sufficiently stained, they were transferred to glycerin for dissection using a Nikon SMZ-8000 stereomicroscope. The appendages were mounted on temporary glycerin slides to facilitate examination using a Nikon Alphaphot-2 YS2 compound microscope with attached drawing tube. Plates were prepared using Adobe Illustrator CC[®]. Body length measurements were taken by measuring the distance from the rostrum to the base of the pleotelson, following the contour of the body using ImageJ software (Abràmoff et al. 2004). The term “defining angle” refers to the posterior margin of the palm and the distal-most point of the posterior margin of the propodus, the area where the tip of the dactylus closes on the propodus. Nomenclature for the terms “seta(e)” and “robust seta(e)” follows published guidelines (Watling 1989), as does the nomenclature of setal types (Zimmer et al. 2009). The term “seta(e)” refers to “simple seta(e)” unless otherwise specified.

DNA extraction and sequencing

Genomic DNA (**gDNA**) was extracted from the digested material prepared during the specimen clearing process. Extractions were performed using Tissue & Insect DNA MiniPrep kits (Zymo Research, Irvine, CA, USA), utilizing a modified protocol (Cannizzaro et al., 2020). Extracted DNA was stored at -20 °C and quantified using a Qubit fluorometer (Invitrogen, Waltham, MA, USA).

Polymerase chain reactions (**PCR**) using primer pairs 18SF/18S1000F and 18S700F/18S1250R (Englisch and Koenemann 2001) amplified ~1000 base pairs (bp)

Table 1. GenBank accession numbers generated as a part of this study.

Original catalog #	Museum #	Species	Locality	Coll Date	Collector(s)	Latitude, Longitude	18S Accession #	28S Accession #	16S Accession #
AGC-1143.1	USNM 1694150	<i>Hyaella plutonia</i> sp. nov.	Spring, ~11 km NNE of Beatty, Nye County, NV, USA	Dec-14-2023	Corey J. Lange	37.00512, -116.72684	PX560105	PX560112	PX560116
AGC-1143.2	USNM 1694151	<i>Hyaella plutonia</i> sp. nov.	Spring, ~11 km NNE of Beatty, Nye County, NV, USA	Dec-14-2023	Corey J. Lange	37.00512, -116.72684	PX560106	PX560113	PX560117
AGC-1124.1	USNM 1694155	<i>Hyaella keepuikantun</i> sp. nov.	Devils Hole II, Nye County, NV, USA	Jan-08-2020	Ambre Chaudoin, Randall Paylor	36.42714, -116.29117	PX560103	PX560110	PX560114
AGC-1125.1	USNM 1694154	<i>Hyaella keepuikantun</i> sp. nov.	Devils Hole II, Nye County, NV, USA	Feb-07-2017	Jeffrey Goldstein, Olin Feuerbacher	36.42714, -116.29117	PX560104	PX560111	PX560115

of nuclear 18S rDNA; 28S_lev2 and 28S_des1 (Verovnik et al. 2005) amplified a ~730-bp segment of the nuclear 28S rDNA gene; and the primer pairs 16StF (Macdonald et al. 2005) and 16SBR (Palumbi et al. 1991) amplified ~350 bp of the mitochondrial 16S rDNA gene. Total PCR volumes of 35 µl contained 30–70 ng of extracted gDNA. PCR master mix contained 17.5 µl GoTaq Master mix (Promega Corporation, Madison, WI, USA), 1 µl of each 10 µM primer, and 13.5 µl of molecular grade water. PCR was performed on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). A negative control lacking only gDNA was included for all sets of PCR reactions to rule out contamination. The following thermal cycler protocols were followed: 18S: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 68.5 °C for 30 s, and 72 °C for 2 min, ending with a 10 min final extension at 72 °C; 28S: 95 °C for 5 minutes, followed by 37 cycles of 94 °C for 45 seconds, 45–53 °C for 30 seconds, and 72 °C for 50 seconds, ending with a 5-minute final extension at 72 °C; 16S: 95 °C for 2 minutes, followed by 37 cycles of 95 °C for 40 seconds, 42–45 °C for 40 seconds, and 72 °C for 1 minute, ending with a 8-minute final extension at 72 °C. PCR products were prepared for sequencing using Exo-CIP Rapid PCR Cleanup (New England Biolabs, Ipswich, MA, USA). Sanger sequencing of purified PCR products was carried out at Eurofins Genomics (Louisville, KY, USA) using industry-standard Sanger sequencing methodology. All sequences generated as a part of this study were submitted to GenBank (Table 1).

Phylogenetic analyses

Pairwise sequence alignment was conducted using MAFFT (Katoh et al. 2002; Katoh and Standley 2013) in GENEIOUS PRIME (www.geneious.com) and checked further by eye. Multiple sequence alignments were performed separately for each gene, accomplished under the same parameters as the pairwise alignments. All markers were concatenated into a final alignment using SEQUENCE MATRIX v.1.8 (Vaidya et al. 2011). We reconstructed phylogenetic relationships with maximum likelihood (ML) analyses using IQTREE v.2.3.6 (Minh et al. 2020). The IQTREE search was run using the MODELFINDER algorithm to select best-fitting substitution

models for individual partitions, which were analyzed under an edge-linked model. For both the 18S and 28S rDNA loci, a TIM model with equal/empirical base frequencies, and a FreeRate model with 2 categories (TIM+F+R2) was selected; for the 16S rDNA, an HKY model with equal/empirical base frequencies, and a gamma distribution with four categories (HKY+F+G4) was selected. Statistical support was estimated using 1,000 ultrafast bootstrap replicates (Minh et al. 2013) and the Shimodaria-Hasegawa approximate likelihood ratio test (Shimodaria and Hasegawa 1999; Guindon et al. 2010). Accession numbers of all individuals included in the phylogenetic analyses are provided in Suppl. material 1.

Results

Molecular phylogenetic analyses

Phylogenetic trees reconstructed here recovered individuals of *Hyalella* from Devils Hole II and the unnamed spring near Beatty as a monophyletic group which also included *H. cretae* (Fig. 2). These populations differed from *H. cretae* at levels similar to those observed in other amphipod taxa (~98–99% similarity in nuclear genes and ~90% in the 16S rDNA), especially in the 18S rDNA, which has demonstrated great utility in identifying species in several amphipod taxa (White 2011; Sisco and Sawicki 2023; Cannizzaro et al. 2025b).

Morphological analyses

Individuals of *Hyalella* examined from the unnamed spring near Beatty and Devils Hole II differed from the other stygomorphic *Hyalella* species in the Nearctic (*H. muerta* and *H. cretae*) in several key morphological characteristics including the shape of the gnathopods, proportions and setation of the basis of pereopod 6, and the armament of the telson and uropod 3 (Table 2). Based on our morphological and molecular results, we describe each of these populations as a new species.

Table 2. Diagnostic morphological characteristics among stygobitic *Hyalella* in the Nearctic. Abbreviations: GN = gnathopod; CPL = carpus posterior lobe.

Character	<i>H. cretae</i>	<i>H. muerta</i>	<i>H. plutonia</i> sp. nov.	<i>H. keepuikantun</i> sp. nov.
Maxilliped inner plate apical robust setae	3	3	2	4
Gn2 (♂) propodus dactylus vertical alignment	well beyond CPL	beyond CPL	beyond CPL	well beyond CPL
Gn2 (♂) palmar margin anterior notch	distinct, posterior angle	distinct, posterior angle	distinct, angled	weak
Pereopod 7 basis posterior margin setae	8	8	6	3
Pereopod 7 basis posterior margin distal-most setae	robust	small/simple	small/simple	small/simple
Pereopod basis projection past ischium	1.5×	1.5×	2.0×	1.1×
Pereopod basis vs. ischium-dactylus	33%	33%	33%	25%
Uropod 3 ramus apex setae	longest robust	longest simple	longest simple	longest robust
Telson apical setae	4	4	2	4
Gn1/2 (♀) palm	concave	straight/convex	straight	straight

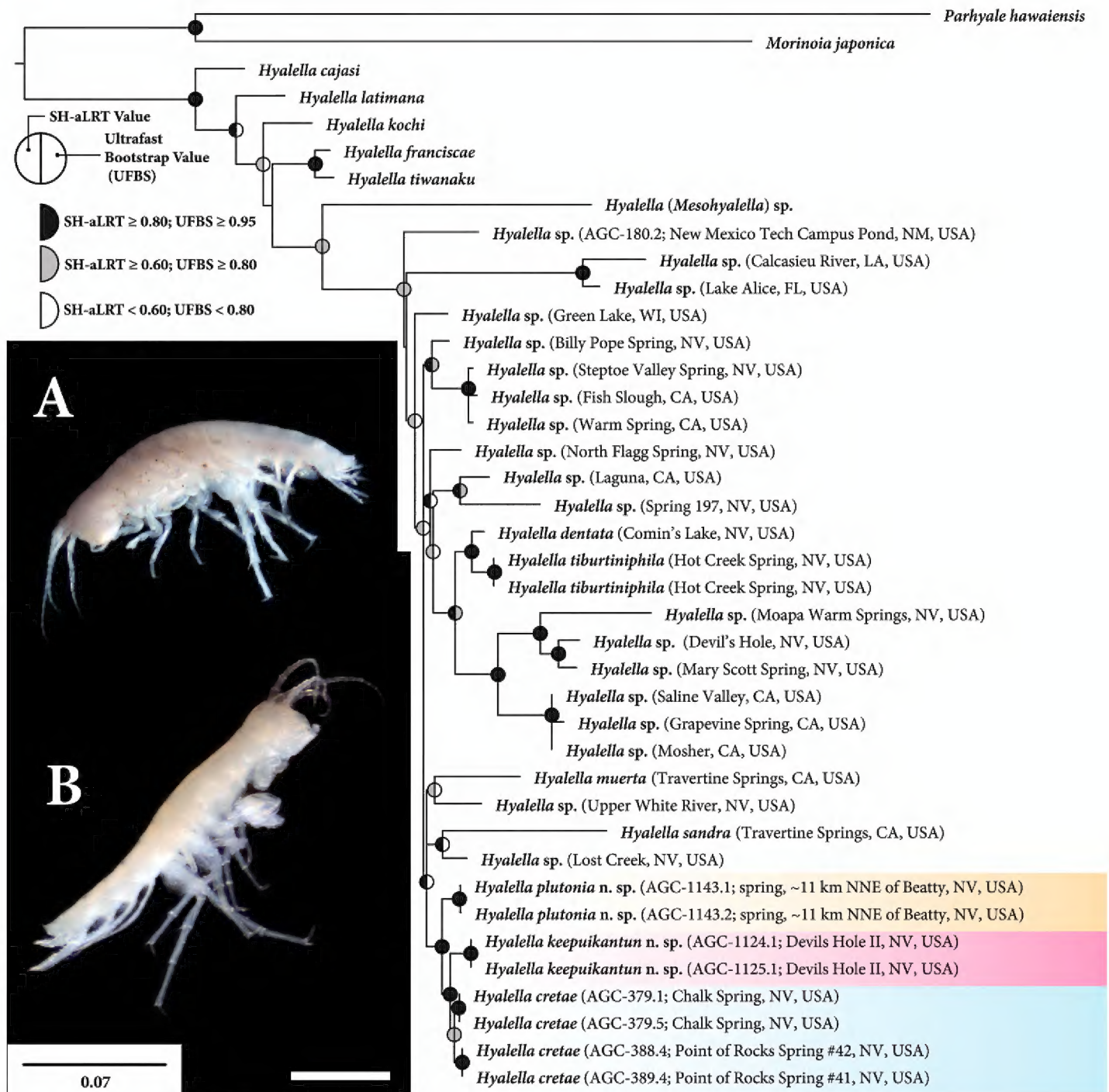


Figure 2. Maximum-likelihood phylogeny reconstructed from the concatenated dataset (18S rDNA, 28S rDNA, 16S rDNA) using IQTREE. Node support for two methodologies is indicated by shaded circles placed on nodes; weakly supported nodes (< 0.40) not marked. Members of the *H. cretae* complex are highlighted by colored gradients. **A** *Hyaella plutonia* sp. nov. female, 3.43 mm, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA **B** *Hyaella keepuikantun* sp. nov. male, 3.15 mm, Devils Hole II, Nye County, Nevada, USA. UFBS, ultrafast bootstrap, SHaLRT, Shimodaira-Hasegawa approximate likelihood ratio test. White scale bar represents 1 mm.

Systematics

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Infraorder Talitrida Rafinesque, 1815

Parvorder Talitridira Rafinesque, 1815

Superfamily Hyaloidea Bulyčeva, 1957

Family Hyaellidae Bulyčeva, 1957

Genus *Hyaella* Smith, 1874

***Hyaella plutonia* Cannizzaro, Lange & Berg, sp. nov.**

<https://zoobank.org/D855A0A5-1777-4CEF-8658-5A513EC4728E>

Figs 3A, B, 4–10

Material examined. • **Holotype**, male 4.20 mm: Unnamed spring, ~0.7 km north of Highway 95 and Oleo Rd intersection, Nye County, Nevada, USA (37.005118, -116.726843); collector: Corey J. Lange, 14 December 2023; USNM 1694150. • **Allotype**, female 3.25 mm: same locality and collection data as holotype; USNM 1694151. • **Paratype** males, n = 2, same locality and collection data as holotype; USNM 1694153. • **Paratype** females, n = 2, same locality and collection data as holotype; USNM 1694152.

Type locality. Unnamed spring, ~0.7 km north of Highway 95 and Oleo Road intersection (on west side of highway), Oasis Valley, Nye County, Nevada, USA (37.005118, -116.726843).

Etymology. The specific epithet *plutonia* is the feminine form of the Latin *Pluto* and the Ancient Greek Πλούτων (Pluton), the god of the Underworld. Pluto's Gates or *ploutonion* were shrines erected to Pluto; one of the most famous is the shrine at Hierapolis, commonly called the "Gateway to the Underworld." This epithet is given both in reference to the hypogean habitat of the species and to play off the nickname of the nearby town of Beatty: "Gateway to Death Valley."



Figure 3. Habitus photographs **A** *Hyaella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **B** *Hyaella plutonia* sp. nov., allotype female, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694151), 3.25 mm **C** *Hyaella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **D** *Hyaella keepuikantun* sp. nov., allotype female, Devils Hole II, Nye County, Nevada, USA (USNM 1694155), 3.62 mm.

Diagnosis. Small-sized, stygomorphic species distinguished from congeners—except members of the *H. muerta* and *H. cretae* complex—by the combination of the following characters: eyes absent; antenna 1 longer than antenna 2; pleonites lacking dorsoposterior carinae; maxilla 1 inner plate with 2 apical pappose setae; male uropod 1 inner ramus lacking long curved setae. Differentiated from *H. muerta* and other members of the *H. cretae* complex by the combination of the following characters: maxilliped inner plate with 2 apical robust setae; male gnathopod 2 palmar margin with distinct anterior notch; pereopod 7 basis projecting $> 1.5 \times$ the length of the ischium, with > 5 posterior setae, all simple, length $> 25\%$ remainder of the pereopod segments; uropod 3 ramus with simple setae; telson with 2 long apical setae.

Description. Holotype male (Figs 3A, 4–8): 4.20 mm in length. Eyes and integumentary pigment absent (Fig. 3A).

Antennae. Antenna 1 (Fig. 4A): Approximately $1.2 \times$ length of antenna 2; peduncle segment 1 lacking posterodistal robust setae; primary flagellum with 12 segments, aesthetascs on distal segments, aesthetascs shorter than respective segments, unpaired. Antenna 2 (Fig. 4B): approximately 30% body length, gland cone distinct; peduncular segment 5 $1.2 \times$ length of segment 4; flagellum with 10 segments.

Mouthparts. Mandibles: left mandible (Fig. 4C): incisor 9-dentate, lacinia mobilis 5-dentate, accessory setal row with 2 plumose setae; molar process well-developed, cylindrical, triturative, lacking seta. Right mandible (Fig. 4D): incisor 5-dentate, lacinia mobilis bifurcate, lobes with numerous fine dentations; accessory setae row with 2 plumose setae; molar similar in form to left mandible.

Upper lip (Fig. 5A): rounded, apical margin of labrum with numerous setules.

Lower lip (Fig. 5B): inner lobes indistinct, outer margin of inner and outer lobes covered in setules; face of lip covered in pubescent setules.

Maxilla 1 (Fig. 5C): inner plate shorter than outer plate, with 2 apical pappose setae, and fine pubescent setules covering the entire plate; outer plate with 9 apical serrate setae, pubescent setules sparse and decreasing laterally and proximally; palp one-segmented, vestigial, 20% length of outer plate, tapering distally, with pubescent setules, palp emerges at slightly over half the length of the outer plate.

Maxilla 2 (Fig. 5D): both inner and outer plates covered in pubescent setules; outer plate subequal in length to inner plate, with numerous apical setae; inner plate narrowing slightly distally with numerous apical setae and 2 pappose facial setae.

Maxilliped (Fig. 5E): inner plate shorter than outer plate with 2 unarmed cuspidate setae along apical margin, surface of plate covered in pubescent setules; outer plate with numerous setae, lacking pubescent setules; palp 4-segmented, second and third segments with numerous marginal, and submarginal setae; dactylus surface lacking comb-scales, nail not elongate.

Gnathopods. Gnathopod 1 (Fig. 6A): coxal plate with 5 short apical setae; basis narrowing proximally, distoposterior surface of segment lacking comb-scales; ischium with a posterior seta; merus with 3 posterior setae, distoposterior surface of segment with sparse comb-scales; carpus subequal in length to propodus, with cluster

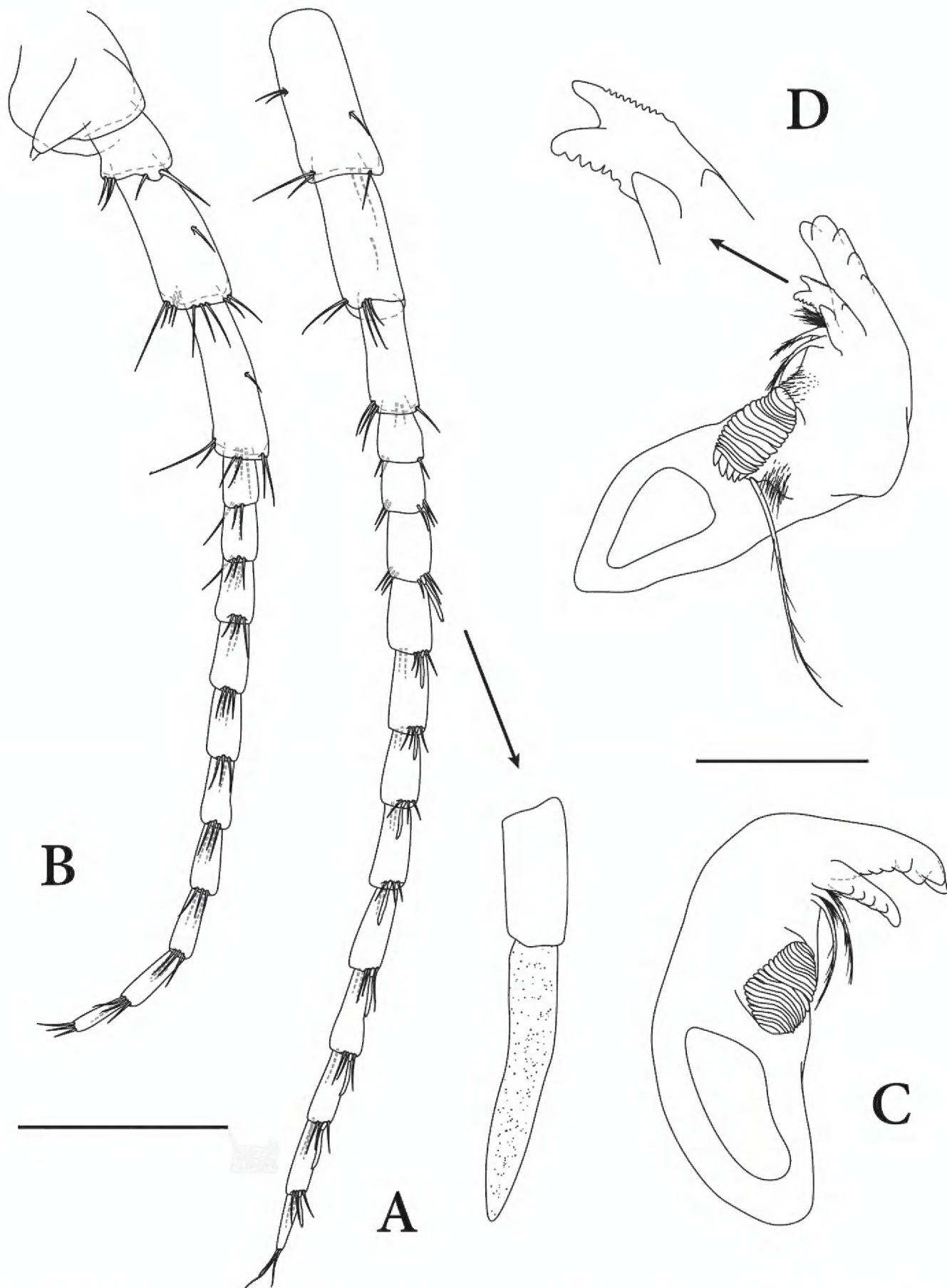


Figure 4. *Hyalella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **A** antenna 1 (single aesthetasc enlarged) **B** antenna 2 **C** left mandible **D** right mandible (lacinia mobilis enlarged). Scale bars: 0.25 mm (**A**, **B**); 0.1 mm (**C**, **D**).

of posterior serrulate setae; propodus $1.4 \times$ longer than broad with 4 inner medial serrulate setae, anterodistal corner with tuft of setae, comb-scales present along posterior margin; palm straight to slightly convex, with 5–9 setae on inner/outer margins; defining angle straight with 2 robust setae; dactylus with plumose seta on outer margin and an inner seta, surface with sparse comb-scales.

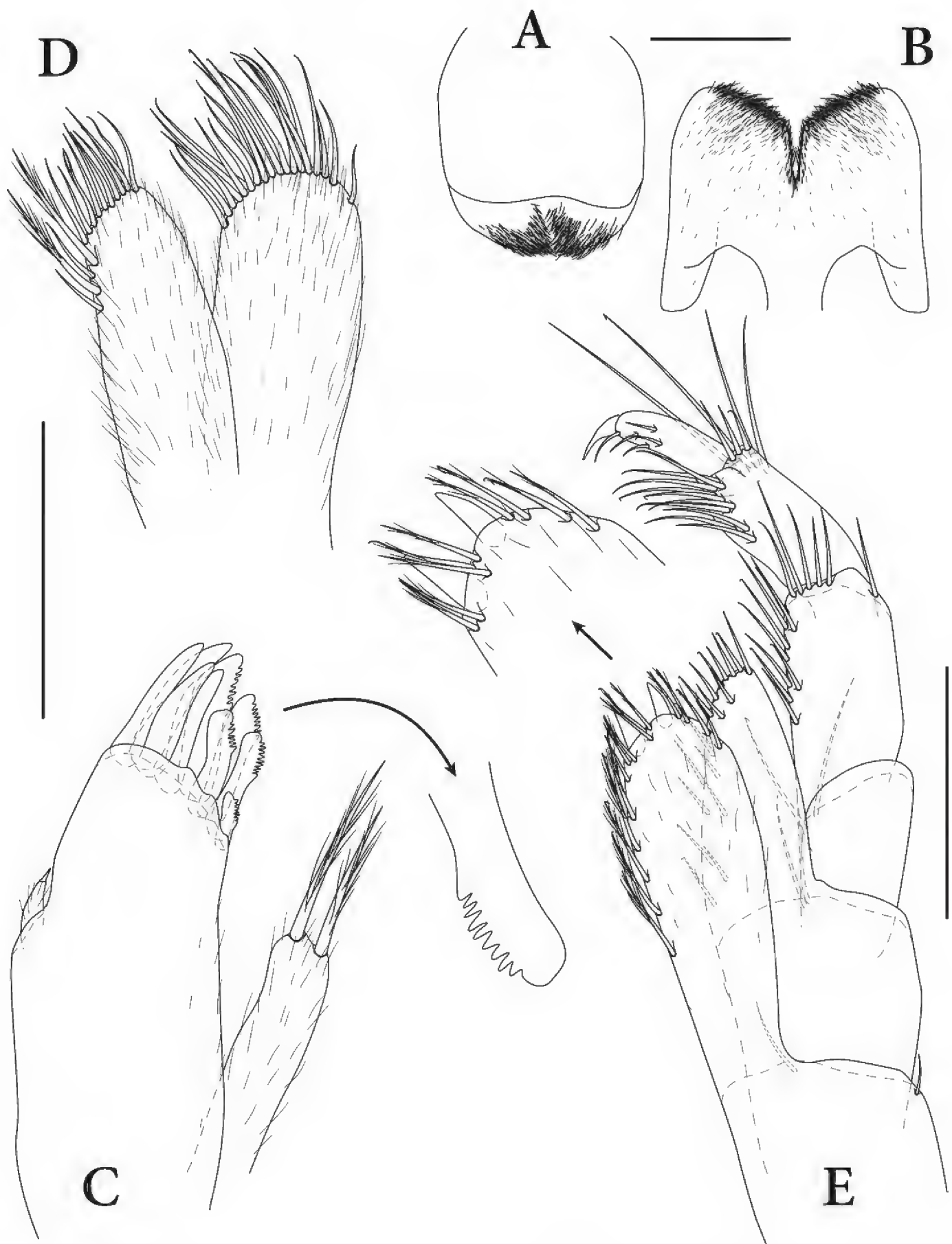


Figure 5. *Hyaella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **A** upper lip **B** lower lip **C** maxilla 1 (outer plate serrate setae enlarged) **D** maxilla 2 **E** maxilliped (apical margin of inner plate enlarged). Scale bars: 0.1 mm.

Gnathopod 2 (Fig. 6B): coxal plate with 8 short apical setae; basis narrowing proximally with 2 posterior setae; ischium with 2 posterior setae; merus with 4 posterior setae, distoposterior surface of segment lacking comb-scales; carpus 33% length of propodus, with 2 anterior setae, posterior margin lobate, 40% breadth of segment, lacking comb-scales; propodus enlarged, powerful, $1.2 \times$ longer than broad, with tuft of

anterodistal setae, posterior setae absent; palm straight, irregular with strong anterior notch, palm with 14 inner and 15 outer robust setae; defining angle expanded, projecting past carpus posterior lobe, with 3 robust setae arranged around notch where tip of dactylus rests, comb-scales extend to base of defining angle; dactylus with plumose seta on outer margin placed medially and 2 inner setae.

Pereopods. Pereopod 3 (Fig. 7A): coxal plate with 6 short apical setae, plate not excavated posteroproximally; basis much longer than coxal plate with a posterior seta; merus $1.3 \times$ longer than carpus; carpus 75% length of propodus; dactylus 50% length of propodus, with plumose seta placed proximally on outer margin and 2 setae inserted adjacent to base of nail.

Pereopod 4 (Fig. 7B): coxal plate with 6 short apical setae, plate excavated posteroproximally; basis much longer than coxal plate with posterior seta; merus $1.3 \times$ longer than carpus; carpus 75% length of propodus; dactylus 50% length of propodus, setation as in pereopod 3.

Pereopod 5 (Fig. 7C): coxal plate large, bilobate, anterior/posterior lobes both with 2 setae each; basis posterior margin weakly convex, with 8 short setae, posterodistal corner convex, anterior margin with 6 robust setae; merus subequal in length to carpus; carpus 80% length of propodus, dactylus 50% length of propodus, with plumose seta placed proximally on posterior margin and 2 setae inserted adjacent to base of nail.

Pereopod 6 (Fig. 7D): coxal plate large, bilobate, with produced posterior lobe; basis posterior margin weakly convex, with 10 shallow serrations paired with short setae, posterodistal corner weakly convex, anterior margin with 6 robust setae; merus subequal in length to carpus; carpus 75% length of propodus; dactylus 35% length of propodus, setation as in pereopod 5.

Pereopod 7 (Fig. 7E): coxal plate diminutive, lobes indistinct; basis posterior margin convex, projecting $2.0 \times$ past the ischium, with 6 shallow serrations paired with short setae extending to start of posterodistal corner, robust setae absent, posterodistal corner straight, anterior margin with 9 robust setae, basis length 33% remainder of pereopod segments; merus 80% length of carpus; carpus 75% length of propodus; dactylus 25% length of propodus, setation like pereopods 5 and 6.

Gills. Coxal gills present on somites 2–7, sternal gills present on somites 3–6 (Figs 6B, 7A–E).

Pleon. Pleosome somites lacking dorsoposterior carinae.

Epimera (Fig. 8A): first epimeron ventral margin unarmed, distoposterior corner not produced, posterior margin with a single seta; second and third epimera like first in form, each with a posterior seta placed more medially.

Pleopods (Fig. 8B): peduncle of pleopod 1 60% length of rami, with 2 coupling hooks, lacking setae; outer and inner rami with 5 and 9 segments respectively. Pleopod 2 peduncle similar to first, with 2 coupling hooks; outer and inner rami with 5, 8 segments respectively. Pleopod 3 peduncle similar to first/second, with 2 coupling hooks; outer and inner rami with 4 and 7 segments respectively.

Urosome. Somites free, bare dorsally. Uropod 1 (Fig. 8C): peduncle $1.5 \times$ length of inner ramus with 5 outer robust setae and an inner seta; rami narrowing slightly distally, outer ramus with 2 marginal robust setae, and 5 apical robust setae; inner ramus 90% length of outer ramus, with 1 marginal robust seta, and 6 apical robust setae.

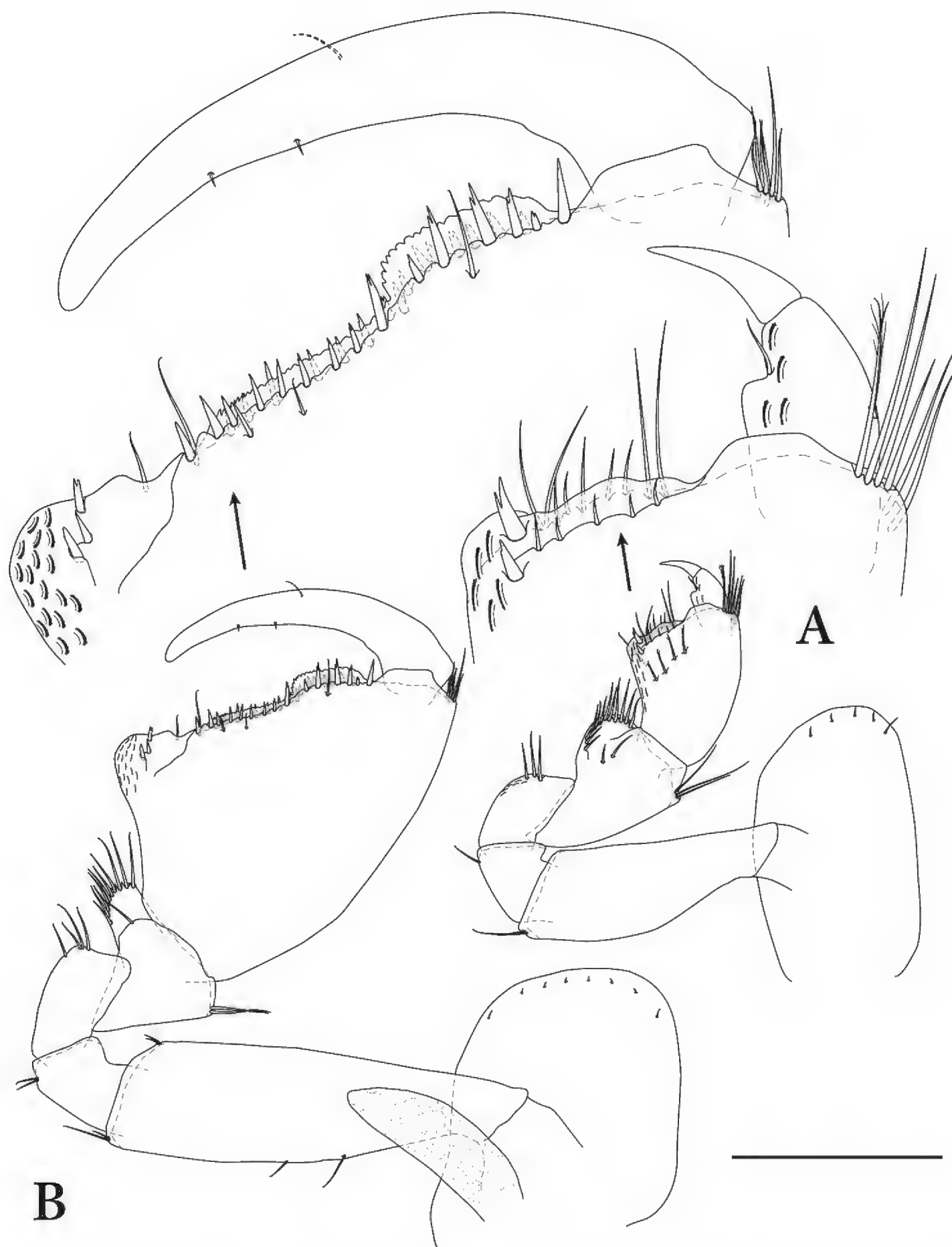


Figure 6. *Hyaella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **A** gnathopod 1 (palmar margin and dactylus enlarged) **B** gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 0.25 mm.

Uropod 2 (Fig. 8D): peduncle 90% length of inner ramus with 1 inner seta and 3 outer robust setae; rami narrowing weakly distally, outer ramus with 2 marginal robust setae, and 5 apical robust setae; inner ramus 1.1 × length of outer ramus, with 1 marginal robust seta, and 6 apical robust setae.

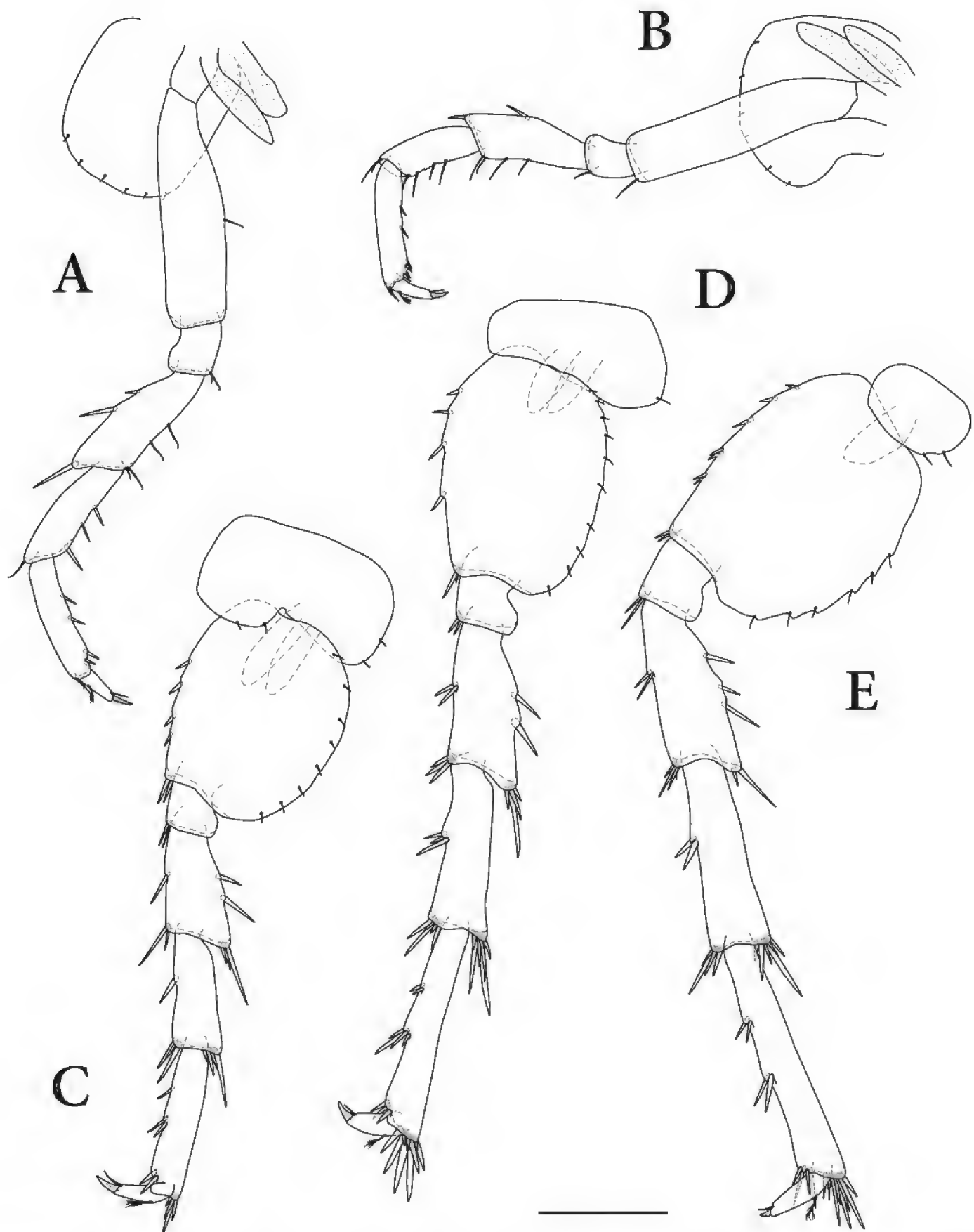


Figure 7. *Hyalella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7. Scale bar: 0.25 mm.

Uropod 3 (Fig. 8E): diminutive, uniramous; peduncle $1.1 \times$ length of ramus, with 2 robust setae; ramus with 3 setae and a robust seta.

Telson (Fig. 8F): fleshy, rounded, not narrowing distally, as long as broad, with 2 unpaired distal setae (one of which with small accessory seta, perhaps regenerated?), 2 triplets of submarginal plumose setae flanking outer margins, apical margin produced, appearing beveled.

Female (Figs 3B, 9, 10): Allotype, USNM 1694151, 3.25 mm in length. Differing from male in presence of weaker gnathopods with weaker carpals/propodi, and minor differences in uropod and telson setation. Structures not described below as in male.

Gnathopods. Gnathopod 1 (Fig. 11A): coxal plate with 5 short apical setae, basis narrowing proximally, lacking marginal posterior setae; ischium with 1 posterior seta; merus with 3 posterior setae, distoposterior surface of segment with sparse comb-scales; carpus 90% length of propodus, with 2 distal setae and cluster of posterior serrulate setae, posterior surface of segment lacking comb-scales; propodus $2.3 \times$ longer than broad with 4 inner medial serrulate setae, anterodistal corner with tuft of setae, comb-scales present along posterior margin; palm straight with 5–8 setae on inner/outer margins; defining angle straight with 2 robust setae; dactylus with plumose seta on outer margin, and 3 inner marginal setae, surface lacking comb-scales.

Gnathopod 2 (Fig. 11B): coxal plate with 5 short apical setae, basis narrowing proximally, lacking marginal posterior setae; ischium with 1 posterior seta; merus with 2 posterior setae, distoposterior surface of segment with sparse comb-scales; carpus 75% length of propodus, posterior margin weakly lobate, lacking comb-scales; propodus $1.6 \times$ longer than broad, with 2 inner medial setae, anterodistal corner with tuft of setae, comb-scales present along both anterior and posterior margins; palm straight with 3–4 setae on inner/outer margins; defining angle with 2 robust setae; dactylus with plumose seta on outer margin, and 3 inner marginal setae, surface covered in comb-scales.

Brood plates. Large setaceous brood plates present on somites 2–5, decreasing in size posteriorly (Fig. 11B).

Urosome. Somites free, bare dorsally. Uropod 1 (Fig. 12A): peduncle $1.3 \times$ length of outer ramus with 3 outer robust setae and an inner robust seta; rami narrowing slightly distally, outer ramus with 3 marginal robust setae, and 4 apical robust setae; inner ramus 90% of outer ramus with 5 apical robust setae, lacking marginal robust setae.

Uropod 2 (Fig. 12B): peduncle subequal to rami, with 1 inner robust seta and 3 outer robust setae; rami not narrowing distally, outer ramus with 1 marginal robust seta, and 5 apical robust setae; inner ramus subequal in length to outer ramus, with identical setation.

Uropod 3 (Fig. 12C): diminutive, uniramous; peduncle 80% length of ramus, with 3 robust setae and a seta; ramus with 2 setae and a robust seta.

Telson (Fig. 12F): fleshy, rounded, not narrowing distally, as long as broad, with 2 unpaired distal setae, 2 pairs of submarginal plumose setae flanking outer margins, apical margin produced, appearing beveled.

Variation. Individuals examined were shown to vary significantly in several morphological characteristics (Table 3).

Distribution and ecology. *Hyaella plutonia* sp. nov. is known only from its type locality, an unnamed rheocene spring which emerges approximately 45 m west of Highway 95, ~0.7 km north of where the highway intersects with Oleo Road. Little is known about either the ecology of *H. plutonia* sp. nov. or the spring which it inhabits. The spring is shallow and silty, emerging near some Fremont cottonwood (*Populus fremontii*) and flowing through primarily broad-leaved cattail (*Typha latifolia*) and yerba mansa (*Anemopsis californica*). This spring occurs on land owned and managed by the

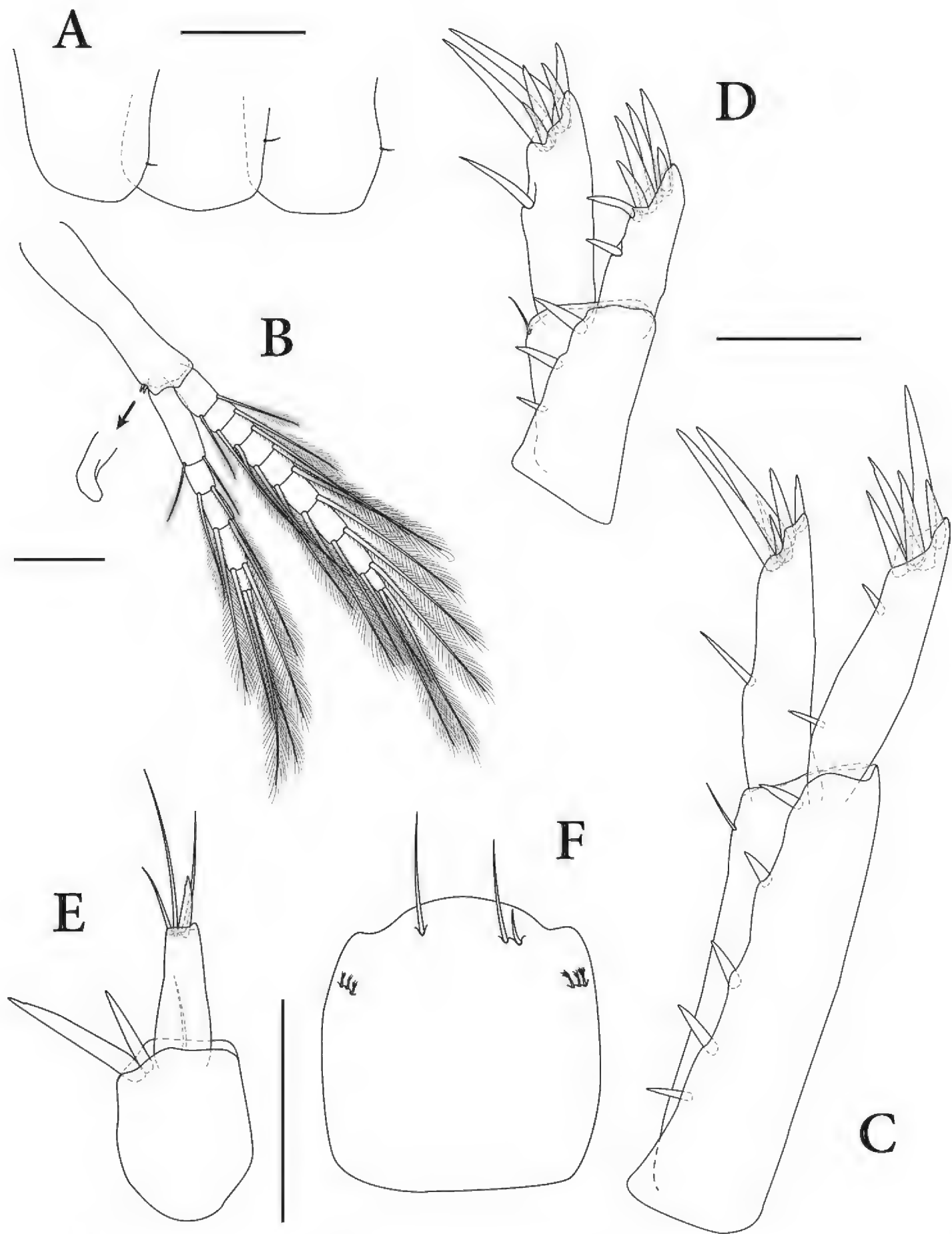


Figure 8. *Hyalella plutonia* sp. nov. holotype male, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694150), 4.20 mm **A** epimera **B** pleopod 1 (coupling spine enlarged) **C** uropod 1 **D** uropod 2 **E** uropod 3 **F** telson. Scale bars: 0.25 mm (**A**, **B**); 0.1 mm (**C**–**E**).

Nevada Department of Transportation (NDOT) and lies 45 m from the edge of Hwy 95. The Federal Highway Administration (FHA) and NDOT are planning to expand and redesignate Hwy 95 to Interstate 11 through this area, which will involve updating the two-lane highway to a separated four-lane interstate. Additionally, groundwater

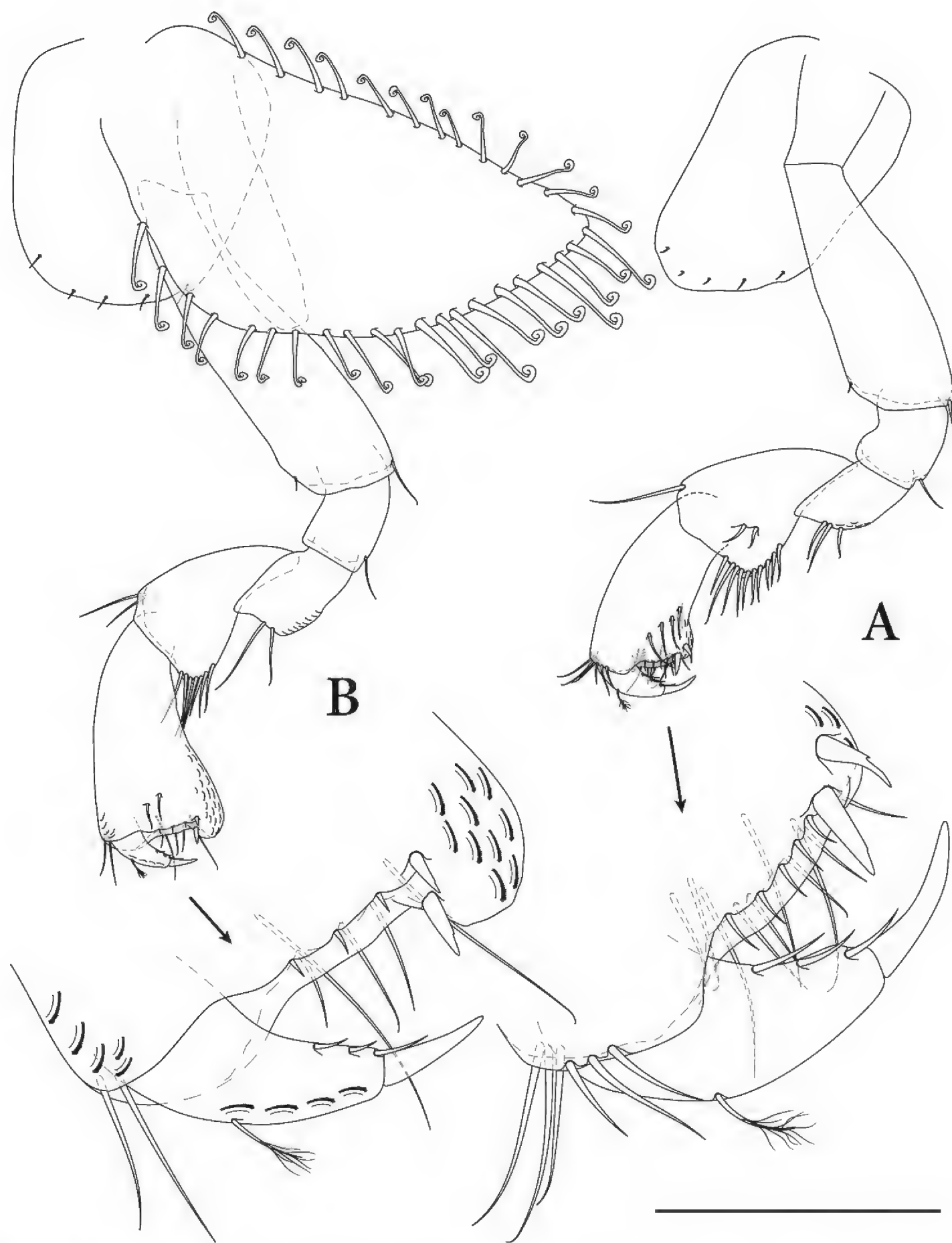


Figure 9. *Hyaella plutonia* sp. nov. allotype female, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694151), 3.25 mm **A** gnathopod 1 (palmar margin and dactylus enlarged) **B** gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 0.25 mm.

withdrawal for the town of Beatty, and proposed and current gold and lithium mines in the immediate area, pose a substantial risk to the continued existence of the type locality spring and *H. plutonia* sp. nov. The type locality spring occurs near the edge of private property where a couple of additional springs occur on the west side of Hwy 95;

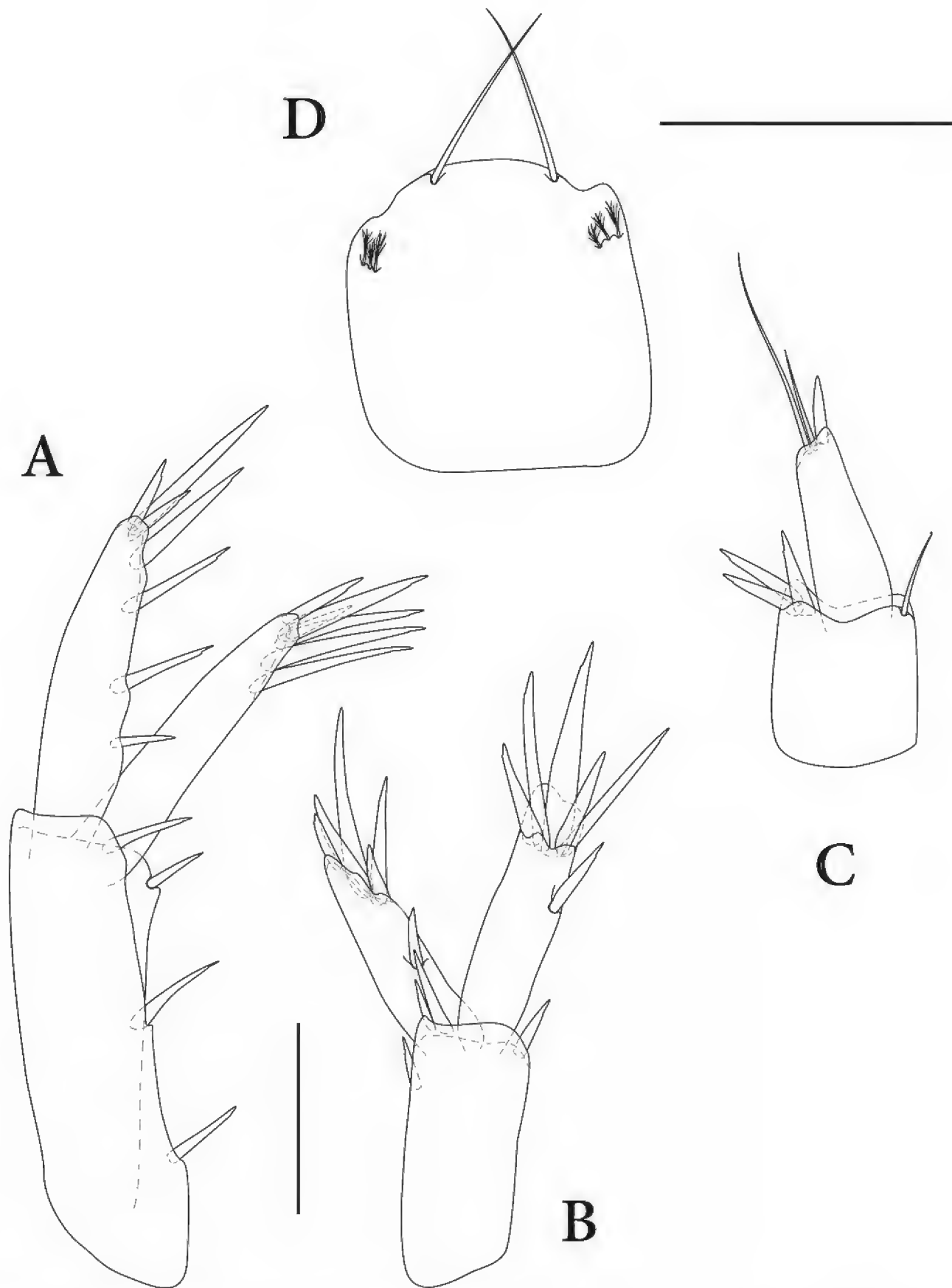


Figure 10. *Hyalella plutonia* sp. nov. allotype female, unnamed spring, ~0.7 km north of Hwy 95 and Oleo Rd, Nye County, Nevada, USA (USNM 1694151), 3.25 mm **A** uropod 1 **B** uropod 2 **C** uropod 3 **D** telson. Scale bars: 0.1 mm.

however, surveys were not conducted on this private property. The second author has surveyed over 20 additional springs in Oasis Valley, on both private and public land, but no other populations of stygobitic hyalellid amphipods have been found; only numerous populations of epigeal hyalellid amphipods have been found. *Hyalella plutonia* sp. nov. co-occurs with the endemic Oasis Valley springsnail (*Pyrgulopsis micrococcus*) and Amargosa toad (*Anaxyrus nelsoni*), which were found during a survey of the spring.

Table 3. Morphological variation observed among individuals of *H. plutonia* sp. nov. and *H. keepuikantun* sp. nov.

Character	Variability (<i>H. plutonia</i> sp. nov.)	Variability (<i>H. keepuikantun</i> sp. nov.)
Body length	~2–4 mm	~2–4 mm
Antenna 1 flagellar segments	9–12	9–10
Antenna 2 flagellar segments	9–11	8–9
Antenna 2 length to body length	1.1–1.3×	1.1–1.5×
Pereopod 7 basis post. setae	6–8	4–5
Pereopod 7 basis post lobe vs ischium length	1.8–2.3×	1.1–1.3×
Uropod 1/2 peduncle inner setae	robust or simple	robust or simple
Uropod 3 ramus setal length	short / long	short / long

***Hyaella keepuikantun* Cannizzaro, Lange & Berg, sp. nov.**

<https://zoobank.org/E2FC2F71-5446-4BF2-9FE6-10B80600D83A>

Figs 11–17

Material examined. • **Holotype**, male 3.48 mm: Devils Hole II, Nye County, Nevada, USA (36.42714, -116.29117); collectors: Jeffrey Goldstein / Olin Feuerbacher, 08 February 2017; USNM 1694154. • **Allotype**, female 3.62 mm: Devils Hole II, Nye County, Nevada, USA (36.42714, -116.29117); collectors: Jeffrey Goldstein / Olin Feuerbacher, 08 January 2020; USNM 1694155. • **Paratype**, male, same locality and collection information as holotype; USNM 1694157. • **Paratype** male, same locality and collection information as allotype; USNM 1694156.

Type locality. Devils Hole II, Death Valley National Park, Nye County, Nevada, USA (36.42714, -116.29117).

Etymology. The specific epithet *keepuikantun* means “blind” in Timbisha Shoshone (literally “no eye having”, kee(no/negative)pui(eye)katün(possessive or having)) is given in reference to the lack of eyes and the location of Devils Hole II within Death Valley, the homeland of the Timbisha Shoshone Tribe. This name was selected in consultation with the Timbisha Shoshone Tribal Historic Preservation Office.

Diagnosis. Small sized, stygomorphic species distinguished from congeners—except members of the *H. muerta* and *H. cretae* complex—by the combination of the following characters: eyes absent; antenna 1 longer than antenna 2; pleonites lacking dorsoposterior carinae; maxilla 1 inner plate with 2 apical pappose setae; male uropod 1 inner ramus lacking long curved setae. Differentiated from *H. muerta* and other members of the *H. cretae* complex by the combination of the following characters: maxilliped inner plate with 4 apical robust setae; male gnathopod 2 palmar margin with weak anterior notch; pereopod 7 basis projecting $< 1.2 \times$ the length of the ischium, with < 5 posterior setae, all simple, length 25% remainder of the pereopod segments; uropod 3 ramus with robust setae; telson with 4 apical setae.

Description. Holotype male (Figs 3C, 11–15): 3.48 mm in length. Eyes and integumentary pigment absent (Fig. 3C).

Antennae. Antenna 1 (Fig. 11A): approximately $1.4 \times$ length of antenna 2; peduncle segment 1 with posterodistal robust seta; primary flagellum with 9 segments,

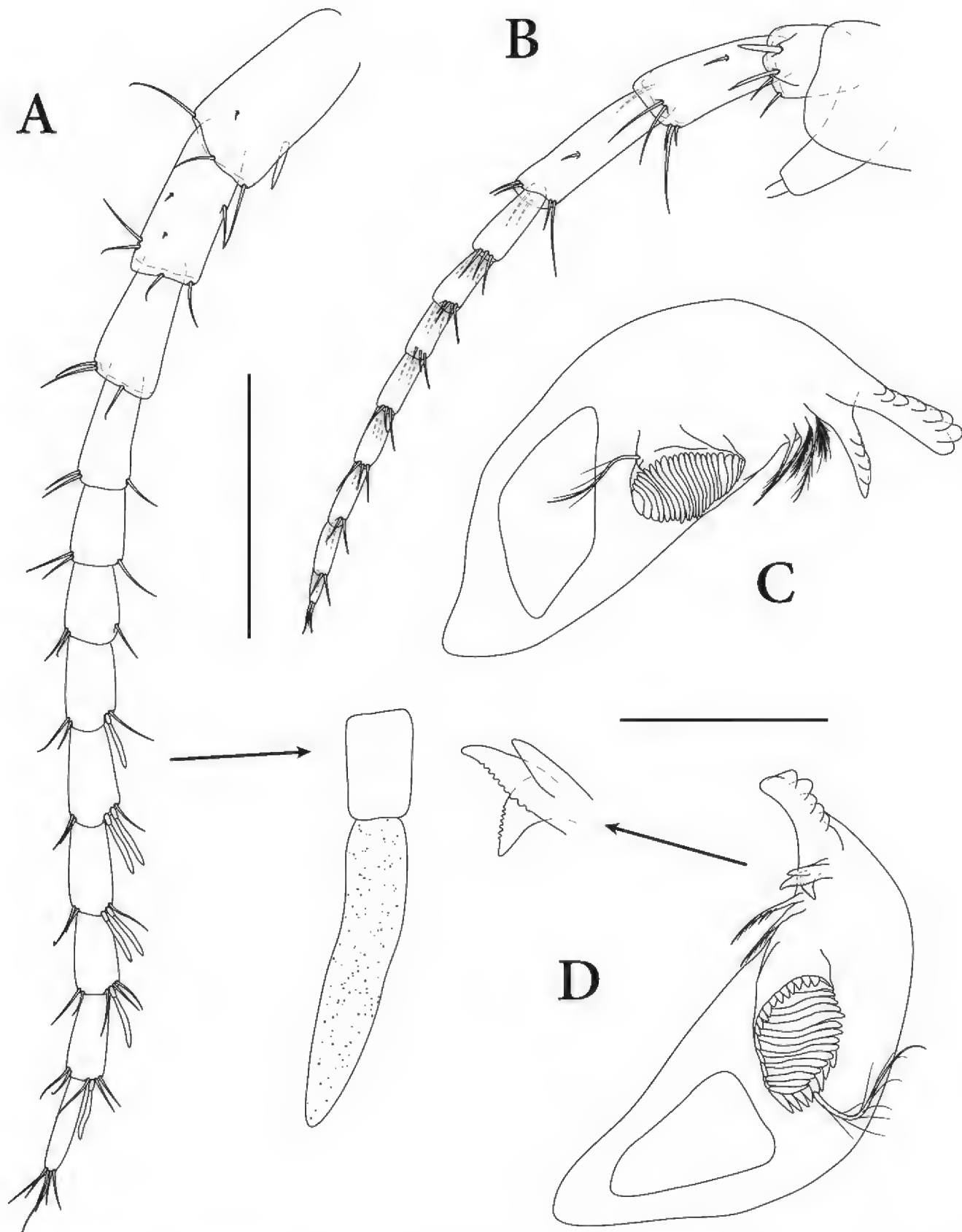


Figure 11. *Hyalella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **A** antenna 1 (single aesthetasc enlarged) **B** antenna 2 **C** left mandible **D** right mandible (lacinia mobilis enlarged). Scale bars: 0.25 mm (**A**, **B**); 0.1 mm (**C**, **D**).

aesthetascs on distal segments, aesthetascs shorter than respective segments, antepenultimate 3 segments with paired aesthetascs.

Antenna 2 (Fig. 11B): approximately 25% body length, gland cone distinct; peduncular segment 5 subequal in length to segment 4; flagellum with 8 segments.

Mouthparts. Mandibles: left mandible (Fig. 11C): incisor 8-dentate, lacinia mobilis 5-dentate, accessory setal row with 3 plumose setae; molar process well-developed, cylindrical triturative, with plumose seta.

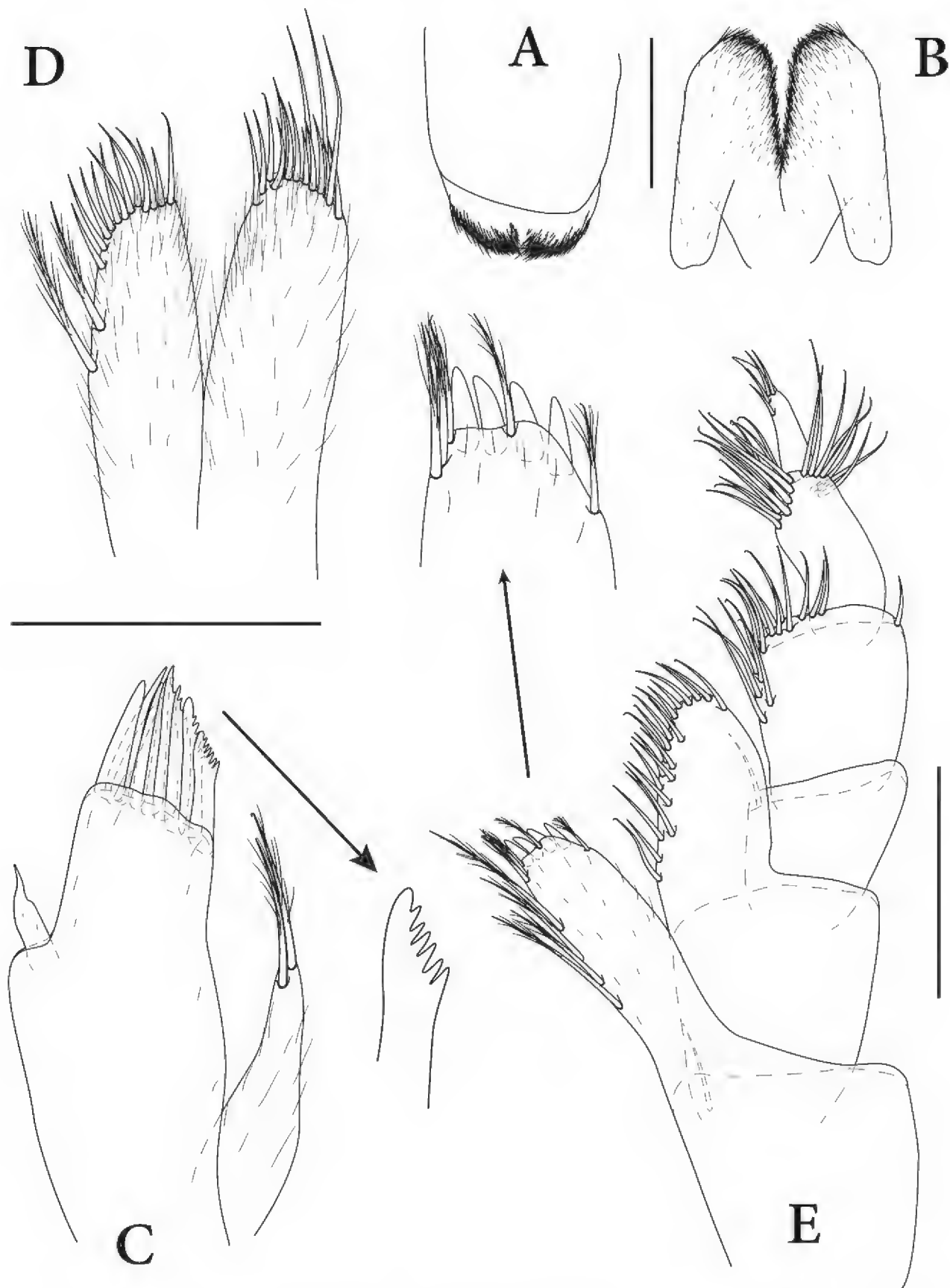


Figure 12. *Hyaella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **A** upper lip **B** lower lip **C** maxilla 1 (outer plate serrate setae enlarged) **D** maxilla 2 **E** maxilliped (apical margin of inner plate enlarged). Scale bars: 0.1 mm.

Right mandible (Fig. 11D): incisor 7-dentate, lacinia mobilis trifid, lobes with numerous fine dentations; accessory setae row with 2 plumose setae; molar similar in form to left mandible.

Upper lip (Fig. 12A): rounded, apical margin of labrum with numerous setules.

Lower lip (Fig. 12B): inner lobes indistinct, outer margin of inner and outer lobes covered in setules; face of lip covered in pubescent setules.

Maxilla 1 (Fig. 12C): inner plate shorter than outer plate, with 2 apical pappose setae, and fine pubescent setules covering the entire plate; outer plate with 9 apical serrate setae, pubescent setules sparse decreasing laterally and proximally; palp one-segmented, vestigial, 20% length of outer plate, tapering strongly distally, with sparse pubescent setules, palp emerges at approximately 70% the length of the outer plate.

Maxilla 2 (Fig. 12D): both inner and outer plates covered in pubescent setules; outer plate subequal in length to inner plate, with numerous apical setae; inner plate narrowing slightly distally with numerous apical setae and 2 pappose facial setae.

Maxilliped (Fig. 12E): inner plate shorter than outer plate with 4 unarmed cuspidate setae along apical margin, surface of plate covered in pubescent setules; outer plate with numerous setae, lacking pubescent setules; palp 4-segmented, second and third segments with numerous marginal, submarginal setae; dactylus surface lacking comb-scales, nail not elongate.

Gnathopods. Gnathopod 1 (Fig. 13A): coxal plate with 4 short apical setae; basis narrowing proximally, distoposterior surface of segment lacking comb-scales; ischium with a posterior seta; merus with 3 posterior setae, distoposterior surface of segment lacking comb-scales; carpus subequal in length to propodus, with cluster of posterior serrulate setae; propodus $1.3 \times$ longer than broad with 5 inner medial serrulate setae, anterodistal corner with tuft of setae, sparse comb-scales present along posterior margin; palm straight, with 5–9 setae on inner/outer margins; defining angle straight with 2 robust setae; dactylus with plumose seta on outer margin and 3 inner setae, surface lacking comb-scales.

Gnathopod 2 (Fig. 13B): coxal plate with 3 short apical setae; basis narrowing weakly proximally; ischium with a posterior seta; merus with 3 posterior setae, distoposterior surface of segment lacking comb-scales; carpus 40% length of propodus, posterior margin lobate, 33% breadth of segment, lacking comb-scales; propodus enlarged, powerful, $1.1 \times$ longer than broad, with tuft of anterodistal setae, posterior setae absent; palm straight, irregular with weak anterior notch, palm with 9 inner and 11 outer robust setae; defining angle weak, projecting slightly past carpus posterior lobe, with 2 robust setae arranged around notch where tip of dactylus rests, comb-scales present apically; dactylus with plumose seta on outer margin placed proximally and 3 inner setae.

Pereopods. Pereopod 3 (Fig. 14A): coxal plate with 5 short apical setae, plate not excavated posteroproximally; basis much longer than coxal plate lacking posterior marginal setae; merus subequal in length to carpus; carpus 80% length of propodus; dactylus 50% length of propodus, with plumose seta placed proximally on outer margin and 2 setae inserted adjacent to base of nail.

Pereopod 4 (Fig. 14B): coxal plate with 4 short apical setae, plate excavated posteroproximally; basis much longer than coxal plate lacking posterior marginal setae; merus subequal to carpus in length; carpus subequal in length to propodus; dactylus 50% length of propodus, setation as in pereopod 3.

Pereopod 5 (Fig. 14C): coxal plate large, bilobate, anterior/posterior lobes both with a seta; basis posterior margin weakly convex, with 4 short setae, posterodistal corner convex, anterior margin with 4 robust setae; merus subequal in length to carpus;

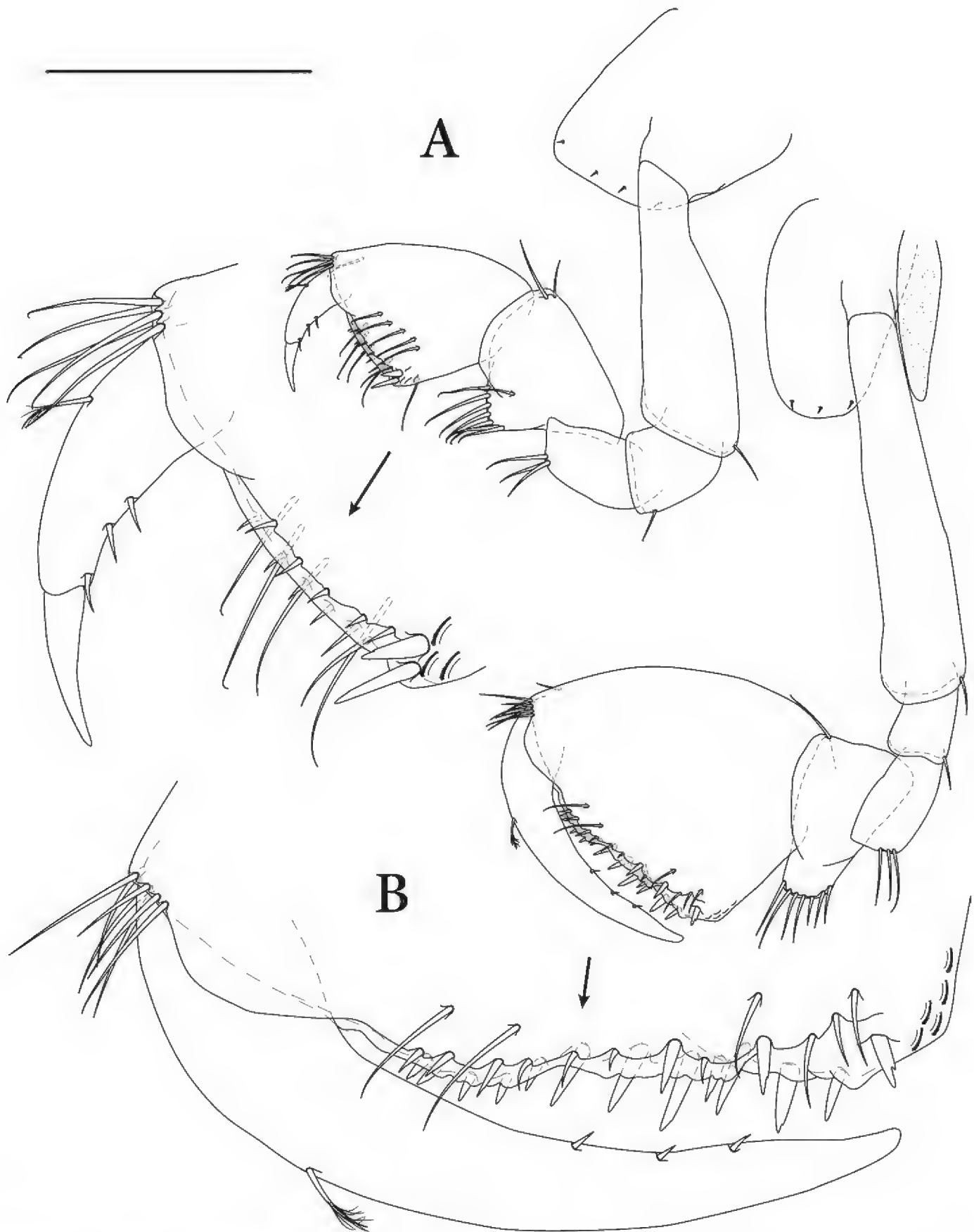


Figure 13. *Hyaella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **A** gnathopod 1 (palmar margin and dactylus enlarged) **B** gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 0.25 mm.

carpus 70% length of propodus, dactylus 40% length of propodus, with plumose seta placed proximally on posterior margin and 2 setae inserted adjacent to base of nail.

Pereopod 6 (Fig. 14D): coxal plate large, bilobate, with produced posterior lobe; basis posterior margin straight to convex, with 3 short setae, posterodistal corner straight, anterior margin with 2 robust setae; merus subequal in length to carpus; carpus 85% length of propodus; dactylus 40% length of propodus, setation as in pereopod 5.

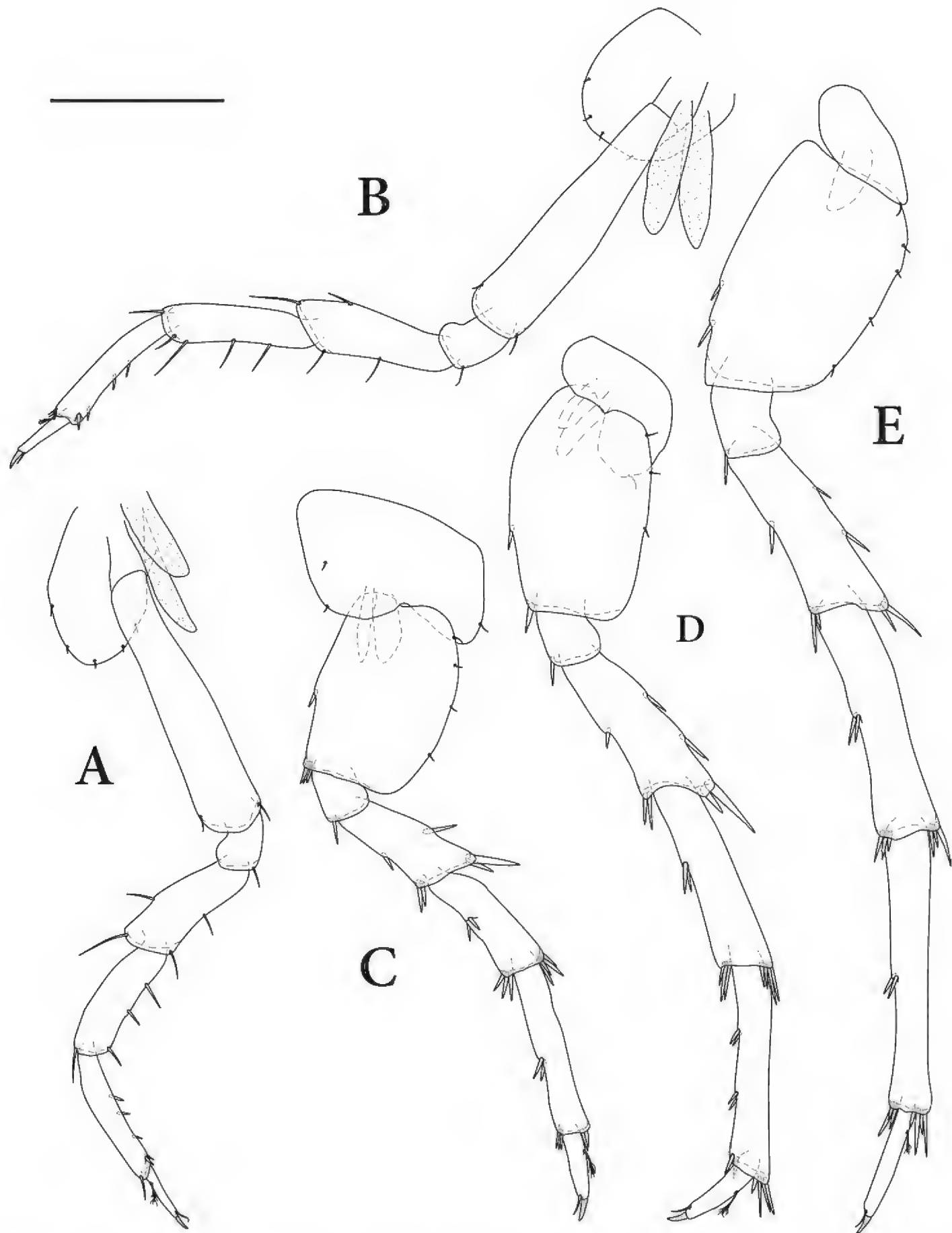


Figure 14. *Hyaella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7. Scale bar: 0.25 mm.

Pereopod 7 (Fig. 14E): coxal plate diminutive, lobes indistinct; basis posterior margin weakly convex, projecting $1.1 \times$ past the ischium, with 4 short setae extending to start of posterodistal corner, robust setae absent, posterodistal corner rounded, anterior margin with 2 robust setae, basis length 25% remainder of pereopod segments;

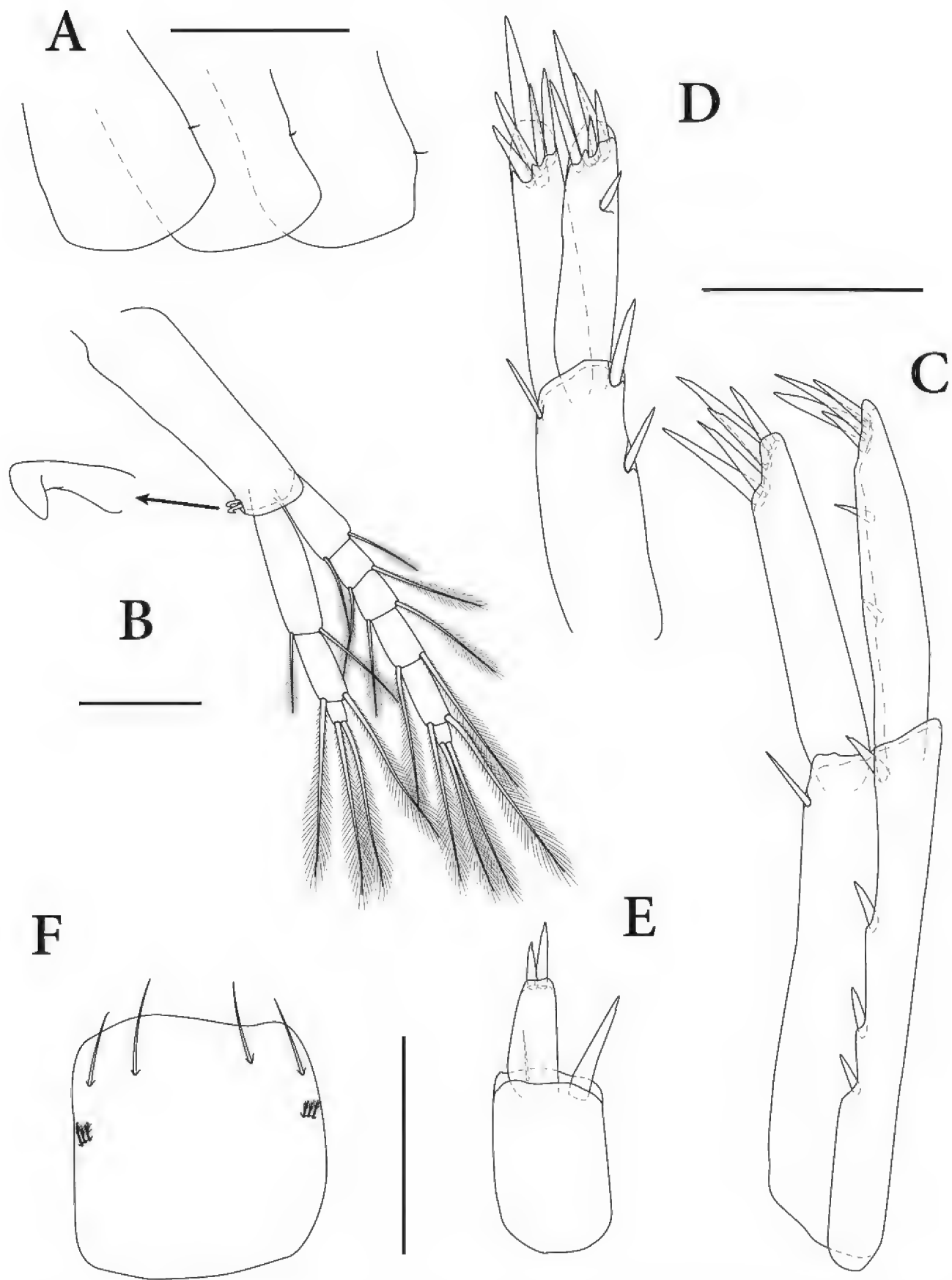


Figure 15. *Hyaella keepuikantun* sp. nov., holotype male, Devils Hole II, Nye County, Nevada, USA (USNM 1694154), 3.48 mm **A** epimera **B** pleopod 1 (coupling spine enlarged) **C** uropod 1 **D** uropod 2 **E** uropod 3 **F** telson. Scale bars: 0.25 mm (**A**, **B**); 0.1 mm (**C**–**E**).

merus 70% length of carpus; carpus 90% length of propodus; dactylus 50% length of propodus, setation like pereopods 5 and 6.

Gills. Coxal gills present on somites 2–7, sternal gills present on somites 3–6 (Figs 13B, 14A–E).

Pleon. Pleosome somites lacking dorsoposterior carinae. Epimera (Fig. 15A): first epimeron ventral margin unarmed, distoposterior corner not produced, posterior

margin with a single seta; second and third epimera like first in form, each with a posterior seta placed more medially.

Pleopods (Fig. 15B): peduncle of pleopod 1 90% length of rami, with 2 coupling hooks lacking setae; outer, inner rami with 3 and 6 segments respectively. Pleopod 2 peduncle similar to first, with 2 coupling hooks; outer, inner rami with 3, 6 segments respectively. Pleopod 3 peduncle similar to first and second, with 2 coupling hooks; outer, inner rami with 3, 5 segments respectively.

Urosome. Somites free, bare dorsally. Uropod 1 (Fig. 15C): peduncle $1.3 \times$ length of inner ramus with 4 outer robust setae and an inner robust seta; rami narrowing slightly distally, outer ramus with 1 marginal robust seta, and 4 apical robust setae; inner ramus 90% length of outer ramus with 5 apical robust setae, marginal robust setae lacking.

Uropod 2 (Fig. 15D): peduncle subequal in length to inner ramus with 1 inner robust seta and 2 outer robust setae; rami not narrowing distally, outer ramus with 1 marginal robust seta, and 5 apical robust setae; inner ramus subequal in length to outer ramus, with 5 apical robust setae, marginal robust setae lacking.

Uropod 3 (Fig. 15E): diminutive, uniramous; peduncle $2 \times$ length of ramus, with a robust seta and a simple seta; ramus with 2 robust setae.

Telson (Fig. 15F): fleshy, rounded, not narrowing distally, as long as broad, with 4 distal setae and 2 triplets of submarginal plumose setae flanking outer margins, apical margin not produced.

Female (Figs 3D, 16, 17): Allotype, USNM 1694155, 3.62 mm in length. Differing from male in presence of weaker gnathopods with weaker carpals/propodi, and minor differences in uropod and telson setation. Structures not described below as in male.

Gnathopods. Gnathopod 1 (Fig. 16A): coxal plate with 5 short apical setae, basis narrowing proximally, lacking marginal posterior setae; ischium with 1 posterior seta; merus with 2 posterior setae, distoposterior surface of segment lacking comb-scales; carpus 90% length of propodus, with a medial seta and a cluster of posterior serrulate setae, posterior surface of segment with comb-scales; propodus $1.2 \times$ longer than broad with 3 inner medial serrulate setae, anterodistal corner with tuft of setae, comb-scales present along posterior margin; palm straight with 5–10 setae on inner/outer margins; defining angle straight with 3 robust setae; dactylus with plumose seta on outer margin, and 3 inner marginal setae, surface lacking comb-scales.

Gnathopod 2 (Fig. 16B): coxal plate with 4 short apical setae, basis narrowing proximally, lacking marginal posterior setae; ischium with 1 posterior seta; merus with 2 posterior setae, distoposterior surface of segment lacking comb-scales; carpus 70% length of propodus, posterior margin lobate, with sparse comb-scales; propodus $1.4 \times$ longer than broad, with 3 inner medial setae, anterodistal corner with tuft of setae, comb-scales present along posterior margin; palm straight with 5–12 setae on inner/outer margins; defining angle with 2 robust setae and 4 additional submarginal robust setae; dactylus with plumose seta on outer margin, and 3 inner marginal setae, surface lacking comb-scales.

Brood plates. (Fig. 16B): large setaceous brood plates present on somites 2–5, decreasing in size posteriorly.

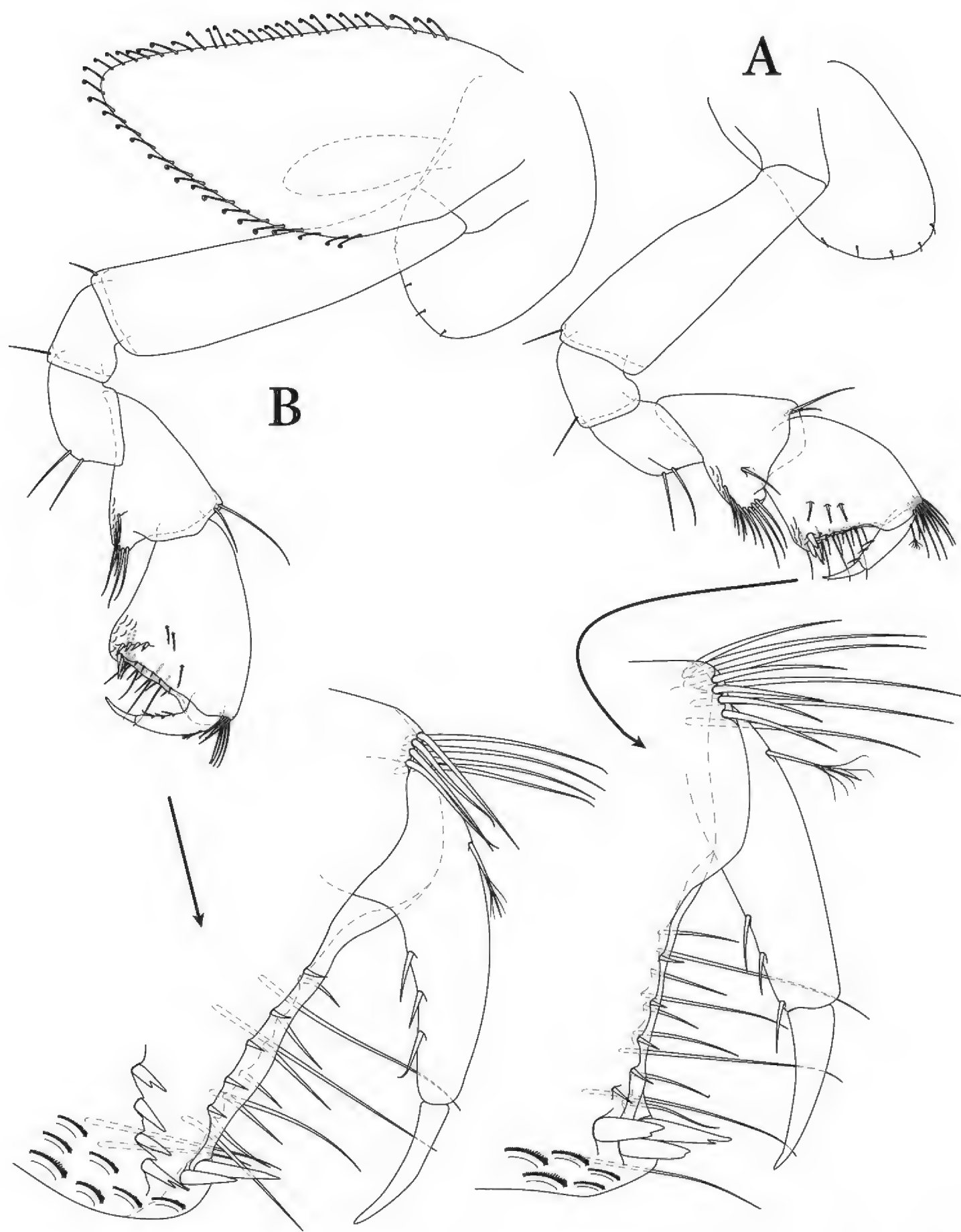


Figure 16. *Hyaella keepuikantun* sp. nov., allotype female, Devils Hole II, Nye County, Nevada, USA (USNM 1694155), 3.62 mm **A** gnathopod 1 (palmar margin and dactylus enlarged) **B** gnathopod 2 (palmar margin and dactylus enlarged). Scale bar: 0.25 mm.

Urosome. Somites free, bare dorsally. Uropod 1 (Fig. 17A): peduncle $1.4 \times$ length of outer ramus with 5 outer robust setae and an inner seta; rami narrowing slightly distally, outer ramus with 3 marginal robust setae, and 4 apical robust setae; inner ramus 90% of outer ramus with 5 apical robust setae, lacking marginal robust setae.

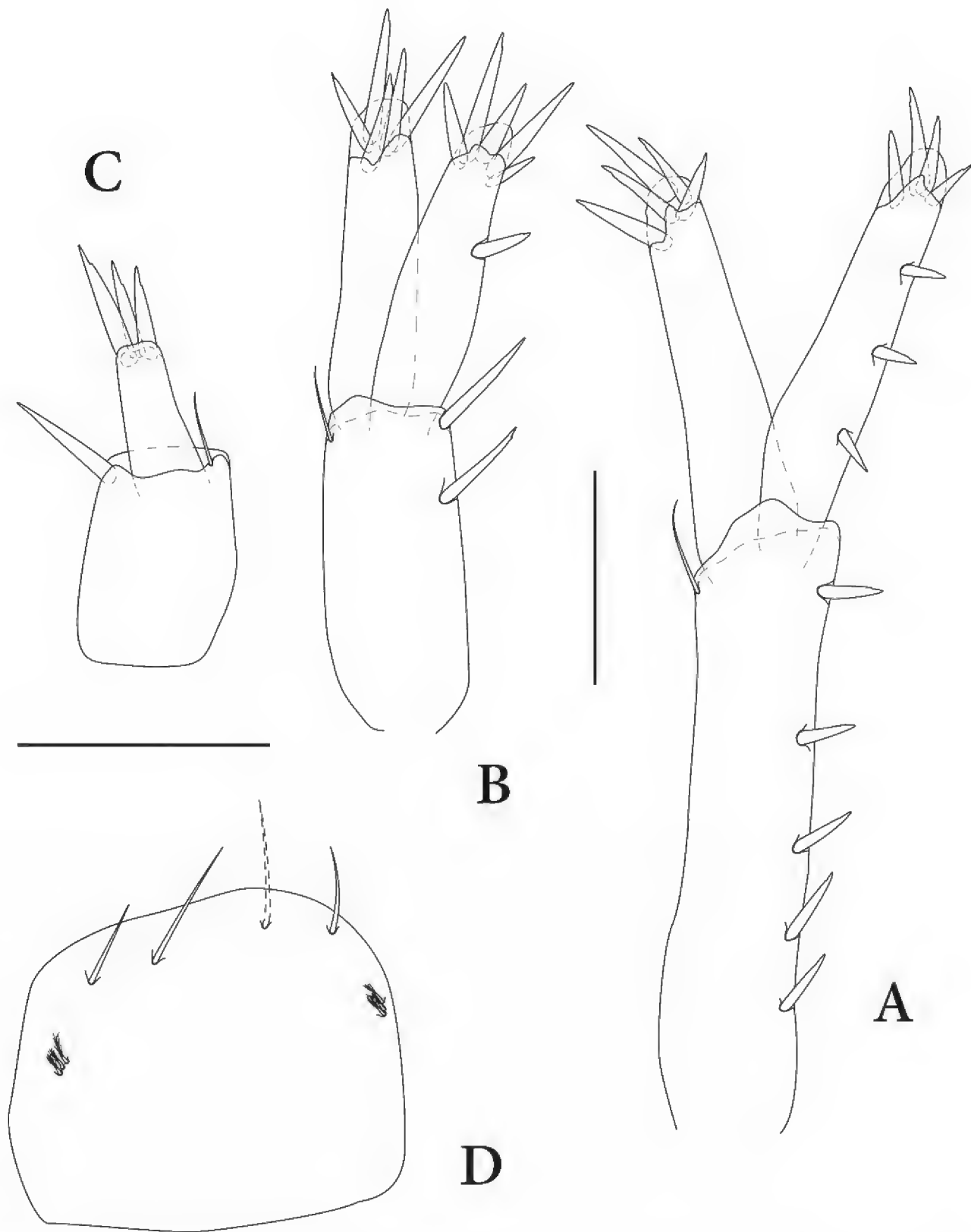


Figure 17. *Hyalella keepuikantun* sp. nov., allotype female, Devils Hole II, Nye County, Nevada, USA (USNM 1694155), 3.62 mm **A** uropod 1 **B** uropod 2 **C** uropod 3 **D** telson. Scale bars: 0.1 mm.

Uropod 2 (Fig. 17B): peduncle subequal to rami, with 1 inner seta and 2 outer robust setae; rami not narrowing distally, outer ramus with 1 marginal robust seta, and 4 apical robust setae; inner ramus subequal in length to outer ramus with 5 apical robust setae, marginal robust setae absent.

Uropod 3 (Fig. 17C): diminutive, uniramous; peduncle $1.5 \times$ length of ramus, with a robust seta and a seta; ramus with 3 robust setae.

Telson (Fig. 17D): fleshy, rounded, not narrowing distally, as long as broad, with 4 distal setae and 3 pairs of submarginal plumose setae flanking outer margins, apical margin not produced.

Variation. Individuals examined were shown to vary significantly in several morphological characteristics (Table 3).

Distribution and ecology. *Hyaella keepuikantun* sp. nov. is known only from its type locality, Devils Hole II (also known as Devils Hole Cave), in Nye County, Nevada, USA. Devils Hole II is located ~200 m north of Devils Hole, and is a separate cave system, manifesting as a small pit opening one meter by half a meter, then opening up to a nearly vertical pit ~40 m deep where it meets the water table (Hoffman 1988). As a result, Devils Hole II is less exposed when compared to its sister site, and epigean species such as the Devils Hole pupfish (*Cyprinodon diabolis*) are absent (Hoffman 1988). An unidentified species of Ostracoda has been found co-occurring with *H. keepuikantun*, but no other known species of aquatic invertebrates have been found in Devils Hole II (Kevin Wilson personal communication). Surveys of the cave have noted water depths of at least 21 meters, but the total depth is likely significantly deeper; its connections to Devils Hole and the rest of the basin remain unknown (Hoffman 1988). *Hyaella keepuikantun* sp. nov. has only been found near the surface of the water in Devils Hole II, where it was collected near bat guano and small, floating, calcite rafts. Devils Hole II is located on a disjunct 16-hectare (40 acre) portion of Death Valley National Park, owned and managed by the National Park Service. Additionally, it is surrounded by Ash Meadows National Wildlife Refuge, managed by the US Fish and Wildlife Service, and the Ash Meadows Area of Critical Environmental Concern (ACEC), managed by the Bureau of Land Management.

Discussion

Our results identify further diversity associated with the “*cretae* complex” of stygobitic *Hyaella* from the Great Basin. Examination of two populations of *Hyaella* from Nye County, Nevada, USA using both molecular and morphological tools identify them as novel species that are separate from the similar *Hyaella cretae* and *Hyaella muerta* (Fig. 2; Table 2). Both *H. plutonia* sp. nov. and *H. keepuikantun* sp. nov. can be readily separated from *H. cretae* and *H. muerta* using a suite of morphological characteristics such as the shape of the palmar margin of gnathopod 2, the setation and shape of the basis of pereopod 7, and the setation of uropod 3 (Table 2). Molecular phylogenies place both new species in a well-supported clade also containing populations of *H. cretae* (Fig. 2). Within this “*H. cretae* complex,” *H. plutonia* sp. nov. appears to be the most basally-derived species, recovered as sister to a clade containing *H. keepuikantun* sp. nov. and *H. cretae* (Fig. 2). Given the distributions of the species, these phyletic relationships are expected (Figs 1, 2). It is likely that the common ancestor to the complex entered groundwater near the current Amargosa River or a similar system, with *H. plutonia* sp. nov. continuing up the river. Contrasting this, the ancestors of *H. keepuikantun* sp. nov. and *H. cretae* likely entered what is now Ash Meadows National Wildlife Refuge. It is possible that these species separated further based on habitat, with *H. cretae* sp. nov. adapting to the shallower rheocrene habitats found at Chalk/Point of Rocks springs while *H. keepuikantun* sp. nov. adapted to the deeper, more open Devils Hole II, which may not be connected to the rest of the system. While *H. cretae* complex

members show similarity to *H. muerta* in many morphological characteristics, including stygomorphic facies, they do not appear to be very closely related (Fig. 2; Cannizzaro et al. 2023). The *H. cretae* complex and *H. muerta* are separated by populations/species occurring in other parts of Nevada, Texas, California and Wyoming, suggestive of a fairly old divergence; observed similarities are likely appearing due to convergence. These results are suggestive of at least two separate groundwater colonization events: (I) the ancestor to *H. muerta* and (II) the ancestor to the *H. cretae* complex.

The discovery of additional hypogean *Hyaletella* species in the northern Mojave Desert is of note. Despite their relative frequency in the neotropics, hypogean *Hyaletella* are restricted in the Nearctic, with all known species occurring in the northern Mojave Desert (Fig. 1; Cannizzaro et al. 2023). Both this restricted distribution and the presence of this genus in groundwater at all are curious, given the widespread prevalence of other groundwater genera such as *Stygobromus*, which are known from hypogean systems from essentially the remainder of the United States and parts of Canada (Holsinger 1986). *Stygobromus* would certainly be the “expected genus” in these systems, given its diversity in California and the surrounding region. The fact that *Hyaletella* has undergone at least two separate colonization events within the region from relatively distant lineages (Fig. 2) suggests that the atypical habitats being occupied by *Hyaletella* spp. may be explained by differences in dispersal abilities of *Hyaletella* versus *Stygobromus* or by local adaptation to such habitats.

The genus *Stygobromus* enjoys a broad distribution in the Nearctic, with over 150 species recorded from a number of hypogean environments ranging throughout much of the eastern and western United States and parts of southern Canada (Holsinger 1986; Horton et al. 2025). In the western United States, *Stygobromus* are most abundant within the Western Cordillera, where along with the cold deserts of the Great Basin and similar regions, a majority of species are recorded (Holsinger 1986; Wang and Holsinger 2001). In addition, with the exception of a single undescribed species from the Chihuahuan Desert in Brewster County, Texas, members of the genus are conspicuously absent from hot, arid regions; no species are recorded from the Mojave or Sonoran deserts (Holsinger 1986; Wang and Holsinger 2001). Furthermore, *Stygobromus* (and, in fact, the entirety of the family Crangonyctidae) is absent in the neotropical realm. In contrast, members of the genus *Hyaletella* enjoy a broader distribution, being found in both the Nearctic and the neotropics. Over 100 species occupy a range of habitats both epigean and hypogean from the Falkland Islands to the arctic tree line of Canada (Horton et al. 2025). As a result, *Hyaletella* have been recorded in all arid regions in North America and it is one of the only epigean freshwater amphipod genera present in most tropical environments within the Neotropical realm (De Los Ríos et al. 2012; Horton et al. 2025). This broad environmental tolerance may also explain *Hyaletella* presence in the northern Mojave. Based on the current distributions of both *Stygobromus* and *Hyaletella* spp., it is likely that high temperatures within the Mojave, Sonoran and other Nearctic hot deserts represent unsuitable habitat for *Stygobromus*, but not *Hyaletella*. This hypothesis suggests that these distributions are due not only to the evolutionary histories of the organisms but characteristics of the region itself, which may represent a faunal break between primarily

temperate *Stygobromus* and primarily neotropical *Hyaella*. Further sampling of hypogean habitats in the southern Mojave and Sonoran deserts has potential to uncover additional diversity among both hypogean *Hyaella* and other subtropical taxa.

Phylogenetic and taxonomic investigations within the genus *Hyaella* have continued to reveal undescribed diversity (Cannizzaro et al. 2023; Cannizzaro et al. 2025a). Our descriptions of *Hyaella plutonia* sp. nov. and *H. keepuikantun* sp. nov. bring the number of described Nearctic species in the genus up to 23 (including *H. azteca* (Sausure 1858)), with five of these endemic to the northern Mojave Desert (Cannizzaro et al. 2025a, 2025b). If species estimates within the genus based on recent phylogenetic studies are to be believed, a significant portion of *Hyaella* in the Nearctic remain undescribed, especially in the Great Basin (Witt et al. 2006; Cannizzaro et al. 2025a). *Hyaella* spp. occupy a very broad range of habitats both in terms of ecology and in terms of geography. Further analyses will not only begin to unravel the taxonomy of the genus—thus decreasing a significant Linnean shortfall—but also further document the unique and varied systems occupied by *Hyaella*. Given that many species within the genus occupy extremely small ranges that are often in highly threatened groundwater-fed ecosystems, like those described here, the conservation implications of additional diversity within the genus is likely to be considerable, with many species occurring in regions where groundwater habitats, and groundwater-fed habitats are imperiled.

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Supplementary material I

GenBank/BOLD accession numbers of sequences utilized in this study

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Data type: xlsx

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